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Reconstitution of the Flavor Signature of Dornfelder Red Wine on the Basis of the Natural Concentrations of Its Key Aroma and Taste Compounds

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ABSTRACT: By application of aroma extract dilution analysis (AEDA) on the volatile fraction isolated from a Dornfelder red wine, 31 odor-active compounds were identified by means of HRGC-MS and comparison with reference compounds. A total of 27 odorants, judged with high FD factors by means of AEDA, was quantitated by means of stable isotope dilution assays, and acetaldehyde was determined enzymatically. In addition, 36 taste-active compounds were analyzed by means of HPLC-UV, HPLC-MS/MS, and ion chromatography. The quantitative data obtained for the identified aroma and taste compounds enabled for the first time the reconstruction of the overall flavor of the red wine. Sensory evaluation of both the aroma and taste profiles of the authentic red wine and the recombinate revealed that Dornfelder red wine was closely mimicked. Moreover, it was demonstrated that the high molecular weight fraction of red wine is essential for its astringent taste impression. By comparison of the overall odor of the aroma recombinate in ethanol with that of the total flavor recombinate containing all tastants, it was shown for the first time that the nonvolatile tastants had a strong influence on the intensity of certain aroma qualities.

KEYWORDS: red wine, sensory analysis, taste, aroma, reconstruction, flavor-matrix interaction

INTRODUCTION

Germany is producing about 1 billion liters of wine compared to a total annual worldwide production of 26.6 billion liters.[1] One third of the German wine production is red wine, with the varietal Dornfelder ranking second.[2] Dornfelder is becoming more and more important, especially in Germany, because the variety is robust, yields well, and produces wines with a rich color.

Although numerous wines have been analyzed, in particular for their volatile composition, the characterization of the key odorants among the bulk of odorless volatiles has scarcely been performed. Guth[3] was the first to identify the key aroma and taste compounds in Scheurebe and Gewürztraminer white wines, also succeeding in a recombinate mimicking the full flavor of both wines on the basis of the concentrations of odorants and tastants occurring in the wines. Further studies applying a similar concept were later performed to mimic a Grenache rosé wine aroma[4] and that of a white wine from Maccabeo.[5]

Although a series of flavan-3-ols and proanthocyanidins have been reported as astringent and bitter-tasting molecules in red wines,[6−8] application of the molecular sensory science approach only recently enabled the elucidation of 82 constituents as taste-active nonvolatiles in red wine by means of a taste dilution analysis.[9] Quantitative analysis of these 82 putative key tastants in Amarone red wine, followed by the determination of their taste threshold concentrations, allowed the ranking of these molecules in their sensory impact by means of dose/activity considerations.[10] On the basis of this preselection, 37 of the 82 compounds were demonstrated to match the overall taste impression of Amarone red wine, thus confirming their importance as key taste molecules. Interestingly, the bitterness of the red wine was induced by subthreshold concentrations of phenolic acid ethyl esters and flavan-3-ols. On the other hand, the velvety astringent onset was imparted by six flavon-3-ol glucosides and dihydroflavon-3-ol rhamnosides. In addition, the puckering astringent offset was caused by a polymeric fraction with molecular weight above 5 kDa, which was found to be amplified by the organic acids, and the perceived sourness was imparted by L-tartaric acid, D-galacturonic acid, acetic acid, succinic acid, L-malic acid, and L-lactic acid and was slightly suppressed by the chlorides of potassium, magnesium, and ammonium. In addition, D-fructose and glycerol as well as subthreshold concentrations of glucose, 1,2-propanediole, and *myo*-inositol were found to be responsible for the sweetness, whereas the mouthfullness and the body of the red wine were induced only by glycerol, 1,2-propanediol, and *myo*-inositol, respectively.

Today, it is well accepted in the literature that nonvolatile constituents may affect the perceived aroma of a given food either by noncovalent binding phenomena or by a cross-modal integration of different sensory inputs on the brain level. Previously, Ferreira et al.[11] suggested a complex formation between volatiles, in particular 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and nonvolatile ingredients in wine. This conclusion supported noncovalent polyphenol/odorant interactions observed before in

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model solutions.[12,13] Also, in a sensory study, it was shown that a simple white wine model matrix, consisting of proteins, polysaccharides, glycerol, and alcohol, influenced the perceived intensity of distinct white wine aroma attributes.[14] By combining extracts isolated from white and red wines, it was recently shown that the release of volatiles was lower from a nonvolatile matrix of red wine as compared to the white wine nonvolatiles.[15]

However, no comprehensive studies have yet been targeted toward the total reconstruction of the flavor of a red wine varietal based on quantitative data of the entire set of volatile key aroma and nonvolatile key taste compounds. Therefore, the aim of this study was to quantify the most important odorants and tastants in a Dornfelder red wine and to perform a flavor recombination study based on the natural concentration of each flavor-active compound.

MATERIALS AND METHODS

Wine. The red wine used for the study was a monovarietal Dornfelder wine (13.0% ethanol by volume, vintage 2004) produced by a German wine grower in the region Rheinhessen (Germany). After grape harvesting, the mash was obtained by pressing the nondestemmed grapes. By adding yeast to the mash it was fermented, and the young wine thus obtained was placed in French oak barrels and stored for 15 months.

Chemicals. The following reference odorants and tastants were obtained from the sources given in parentheses: 2,3-butanedione, butanoic acid, (4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one, decanoic acid, ethyl butanoate, ethyl hexanoate, (*S*)-ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 4-hydroxy-3-methoxybenzaldehyde, (*S*)-2-methyl-1-butanol, 3-methyl-1-butanol, (*S*)-2-methylbutanoic acid, 3-methylbutanoic acid, 2-methylpropanoic acid, 3-(methylthio)propanal, 5-pentyldihydrofuran-2(3*H*)-one, 2-phenylacetic acid, 2-phenylethanol, 2-phenylethyl acetate, (*E*)-aconitic acid, (*Z*)-aconitic acid, caffeic acid, calcium L-lactate pentahydrate, (+)-catechin, *p*-coumaric acid, (−)-epicatechin, D-fructose, furan-2-carboxylic acid, galacturonic acid monohydrate, gallic acid, glycerol, D-glucose, lactic acid, magnesium acetate tetrahydrate, malic acid, L-proline, protocatechuic acid ethyl ester, succinic acid, syringic acid, and vanillic acid (Sigma-Aldrich, Taufkirchen, Germany); 4-allyl-2-methoxyphenol, acetic acid, citric acid, ethyl methylpropanoate, 2-methoxyphenol, ammonium acetate, ethanol absolute, potassium hydroxide, sodium acetate, and sodium hydroxide (Merck, Darmstadt, Germany); 4-ethyl-2-methoxyphenol (Lancaster, Mühlheim am Main, Germany); caftaric acid and L-(+)-tartaric acid (Carl Roth, Karlsruhe, Germany); isorhamnetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-galactopyranoside, and syringetin-3-*O*-β-D-glucopyranoside (Extrasynthese, Genay Cedex, France); L-proline ($^{13}C_5$, ^{15}N) (Cambridge Isotope Laboratories, Inc., Andover, MA); caffeic acid ethyl ester (ABCR Chemicals, Karlsruhe, Germany); and gallic acid ethyl ester (Acros Organics, Geel, Belgium). (*E*)-1-(2,6,6-Trimethylcyclohex-1-en-1-yl)but-2-en-1-one was a gift from Symrise (Holzminden, Germany). [$^2H_3$]-Acetic acid and [$^{13}C_2$]-2-phenylacetic acid were from Sigma-Aldrich. Solvents were of HPLC or LC-MS grade (J. T. Baker, Deventer, The Netherlands), and water for chromatographic separations was purified with a Milli-Q Advantage A10 system (Millipore, Molsheim, France).

The following reference compounds were prepared according to the literature given in the Supporting Information: [$^{13}C_4$]-2,3-butanedione, [$^2H_2$]-butanoic acid, [$^2H_2$]-(4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one, [$^2H_2$]-decanoic acid, [$^2H_3$]-ethyl butanoate, [$^2H_3$]-ethyl hexanoate, [$^2H_3$]-4-ethyl-2-methoxyphenol, [$^2H_5$]-ethyl 2-methylbutanoate, [$^2H_3$]-ethyl 3-methylbutanoate, [$^2H_3$]-ethyl methylpropanoate, [$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, [$^{13}C_2$]-4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, [$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde, [$^2H_3$]-2-methoxyphenol, [$^2H_2$]-3-methyl-1-butanol, [$^2H_2$]-3-methylbutanoic acid, [$^2H_3$]-3-(methylthio)propanal, [$^2H_2$]-5-pentyldihydrofuran-2(3*H*)-one, [$^{13}C_2$]-2-phenylethanol, [$^{13}C_2$]-2-phenylethyl acetate, [$^2H_{2-4}$]-4-propyl-2-methoxyphenol, [$^2H_{4-6}$]-(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one, vescalagin, castalagin, and *p*-coumaric acid ethyl ester.

Isolation of Volatiles. NaCl (10 g) was added to an aliquot (100 mL) of the red wine, the solution was extracted with diethyl ether (3 × 100 mL), and the combined organic phases were washed with brine and finally dried over anhydrous sodium sulfate. After filtration and concentration on a Vigreux column to ∼100 mL, the volatiles were isolated by means of the solvent-assisted flavor evaporation (SAFE) technique. To separate the acidic from the neutral−basic volatiles, the distillate was treated with aqueous sodium carbonate (0.5 mol/L, 3 × 50 mL) to yield the fraction of the neutral−basic volatiles (NBF) in the ethereal solution. The combined aqueous layers were adjusted to pH 2 with hydrochloric acid (16% in water) and extracted with diethyl ether (3 × 70 mL) to obtain the acidic volatiles (AF). Fractions AF and NBF were both concentrated to about 3 mL at 40 °C using a Vigreux column (60 cm × 1 cm) and, then, further concentrated to 1 mL using a microdistillation apparatus.

High-Resolution Gas Chromatography−Olfactometry (HRGC-O). HRGC-O was performed using a gas chromatograph type 5160 Mega series (Carlo Erba Instruments) (Milano, Italy) using the following fused silica capillaries: FFAP and DB-5 (both 30 m × 0.32 mm, 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The samples were applied by the cold on-column technique at 40 °C using helium at a flow rate of 2.2 mL/min as the carrier gas. For the FFAP capillary, the initial temperature of 40 °C was held for 2 min and then raised at 6 °C/min until 230 °C. For the DB-5 capillary, the initial temperature of 40 °C was held for 2 min and then raised at 6 °C/min until 250 °C.

For HRGC-O, the effluent was split 1:1 by volume at the end of the capillary by means of a Y-type glass splitter and two deactivated fused silica capillaries (50 cm × 0.25 mm). One part was directed to the FID held at 240 °C, and the other part to a heated sniffing port (190 °C). Calculation of linear retention indices (RI) was done using a series of *n*-alkanes as described previously.[16]

Aroma Extract Dilution Analysis (AEDA). In a first approach, the effluent of the volatiles present in the undiluted samples (AF and NBF) was evaluated by four sniffers to eliminate potential gaps in detecting odor-active regions. Then, the flavor dilution (FD) factors of the odor-active compounds were determined by diluting the extract stepwise 1:1 (v/v) with diethyl ether and by analyzing each dilution by HRGC-O.[17] By definition, the FD factor obtained for each odorant in the AEDA is equal to the highest dilution in which the odorant can be perceived at the sniffing port.

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). For compound identification, mass spectra were generated by means of a sector field mass spectrometer Finnigan type MAT 95 S (Bremen, Germany) in the electron impact (EI) mode at 70 eV.

Determination of the Concentrations of Labeled Internal Standards. Because most of the syntheses were performed at a microscale level, common purification procedures, such as distillation or crystallization, could not be applied. To determine the exact concentrations, the following approach was used: First, a response factor was determined by HRGC analysis (FID) of a solution containing defined amounts of the respective unlabeled compound and methyl octanoate as a reference standard. In a second step, a defined amount of methyl octanoate was added to a defined volume of the solution containing the labeled compound. The resulting mixture was analyzed by HRGC-FID and the concentration of the labeled compound was calculated from the

Table 1. Isotopically Labeled Standard, Ions Selected, and Response Factors Used in the Isotope Dilution Assays of the 27 Red Wine Aroma Compounds

odorant	ion (m/z)	labeled internal standard	ion (m/z)	RF[a]
4-allyl-2-methoxyphenol	165	[$^{2}H_{2-4}$]-4-propyl-2-methoxyphenol	167−169[b]	1.0
2,3-butanedione	87	[$^{13}C_4$]-2,3-butanedione	91	0.99
butanoic acid	89	[$^{2}H_2$]-butanoic acid	91	0.93
(4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one	157	[$^{2}H_2$]-(4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one	159	0.72
decanoic acid	173	[$^{2}H_2$]-decanoic acid	175	0.85
acetic acid	61	[$^{2}H_3$]-acetic acid	64	0.89
ethyl butanoate	117	[$^{2}H_3$]-ethyl butanoate	120	1.0
ethyl hexanoate	145	[$^{2}H_3$]-ethyl hexanoate	148	1.0
4-ethyl-2-methoxyphenol	153	[$^{2}H_3$]-4-ethyl-2-methoxyphenol	156	0.89
(*S*)-ethyl 2-methylbutanoate	131	[$^{2}H_5$]-ethyl 2-methylbutanoate	136	1.0
ethyl 3-methylbutanoate	131	[$^{2}H_3$]-ethyl 3-methylbutanoate	134	1.0
ethyl methylpropanoate	117	[$^{2}H_3$]-ethyl methylpropanoate	120	1.0
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	129	[$^{13}C_2$]-3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	131	1.0
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	129	[$^{13}C_2$]-4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	131	0.82
4-hydroxy-3-methoxybenzaldehyde	153	[$^{2}H_3$]-4-hydroxy-3-methoxy-benzaldehyde	156	1.0
2-methoxyphenol	125	[$^{2}H_3$]-2-methoxyphenol	128	0.96
(*S*)-2- and 3-methyl-1-butanol	71	[$^{2}H_2$]-3-methyl-1-butanol	73	0.90
(*S*)-2- and 3-methylbutanoic acid	103	[$^{2}H_2$]-3-methylbutanoic acid	105	0.88
2-methylpropanoic acid	89	[$^{2}H_2$]-butanoic acid	91	0.68
3-(methylthio)propanal	105	[$^{2}H_3$]-3-(methylthio)propanal	108	1.0
5-pentyldihydrofuran-2(3*H*)-one	157	[$^{2}H_2$]-5-pentyldihydrofuran-2(3*H*)-one	159	0.72
2-phenylacetic acid	137	[$^{13}C_2$]-2-phenylacetic acid	139	0.95
2-phenylethanol	105	[$^{13}C_2$]-2-phenylethanol	107	1.0
2-phenylethyl acetate	105	[$^{13}C_2$]-2-phenylethyl acetate	107	1.0
(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one	191	[$^{2}H_{4-6}$]-(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one	195−197[b]	0.95

[a] MS response factor determined by analyzing defined mixtures of the analyte and the internal standard. [b] Internal standard was used as a mixture of isotopologues.

peak areas of the gas chromatogram, using the FID response factor determined for the unlabeled compound.

Quantitative Analysis of Odorants by Means of Stable Isotope Dilution Assays and High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). Various amounts of wine (1−300 mL) were used for quantitation, depending on the amounts of the target compounds estimated in preliminary experiments. After addition of the labeled standards (resulting in a concentration of 1−5 μg/mL of each compound in the extract), the wine samples were equilibrated for 30 min and then extracted with diethyl ether. The isolation of the volatiles was performed as described above.

Quantitations were performed using two different HRGC-MS systems equipped with either a 30 m × 0.32 mm, 0.25 μm, FFAP or a 30 m × 0.32 mm, 0.25 μm, DB-5 column and also a 30 m × 0.25 mm, 0.32 μm, OV-1701 capillary (all J&W Scientific, Folsom, CA). Acetic acid and butanoic acid as well as (*S*)-2- and 3-methylbutanoic acid were quantified using a Varian GC 3800 gas chromatograph (Varian, Darmstadt, Germany) coupled to a Saturn 2000 ion trap mass spectrometer (Varian). Quantitation of the remaining compounds was performed by means of a two-dimensional HRGC-MS system consisting of a Trace 2000 series gas chromatograph (Thermo Quest, Egelsbach, Germany) coupled to a Varian GC 3800 gas chromatograph. Mass spectra were recorded in the chemical ionization (CI) mode with methanol as the reagent gas using the Varian Saturn 2000 mass spectrometer. For each compound, a calibration factor was calculated by analyzing mixtures of defined amounts of the labeled and unlabeled compound in three different mass ratios (1:3, 1:1, 3:1). The MS response factors determined are summarized in Table 1.

Quantitation of Acetaldehyde. Acetaldehyde was quantified enzymatically using a UV-test kit (R-Biopharm, Darmstadt, Germany).

Determination of Isomeric Distributions. The enantiomeric ratios in ethyl 2-methylbutanoate and 2-methylbutanoic acid were determined by two-dimensional GC-MS using the chiral BGB-176 (30 × 0.25 mm, 0.25 μm film thickness) (BGB Analytik AG, Anwil, Switzerland) capillary as the second column.[18] Separation of the 2-methyl-1-butanol enantiomers and 3-methyl-1-butanol was achieved using a 30 mm × 0.25 mm, 0.25 μm, BGB 174-E stationary phase (BGB Analytik AG). For the differentiation of 2- and 3-methylbutanoic acid, first the sum of both acids was determined by a stable isotope dilution assay. Then, the ion intensities of the mass fragments m/z 74 for 2-methylbutanoic acid and m/z 60 for 3-methylbutanoic acid were monitored by MS-EI, and the concentrations of both acids were separately calculated using a calibration curve prepared by analyzing defined mixtures of both isomers.

Quantitative Analysis of Taste-Active Compounds by Means of LC-MS-MS. Mass spectrometric analysis was performed by electrospray ionization (ESI) using an API 4000 Q-Trap LC-MS-MS system (ABSciex Instruments, Darmstadt, Germany), connected to an Agilent 1200 series HPLC system (Agilent, Karlsruhe, Germany). The ion-spray voltage was set at −4500 V in the ESI^- mode and at +5500 V in the ESI^+ mode. Zero grade air served as nebulizer gas (45 psi) and as turbo gas (400 °C) for solvent drying (55 psi). Nitrogen served as the curtain gas (20 psi) as well as collision gas (4.5×10^{-5} Torr). The MS-MS parameters, declustering potential (DP), entrance potential (EP), collision cell entrance potential (CEP), collision energy (CE), and cell exit potential (CXP) were tuned for each individual compound by flow

injection (20 μL/min), detecting the fragmentation of the $[M + H]^+$ or $[M - H]^-$ molecular ions into specific product ions after collision with nitrogen (4.5×10^{-5} Torr). Analysis of mass spectrometric data was performed using Analyst software v 1.5.

Flavonol Glycosides. Quantitative analysis was performed following the procedure reported previously,[10] that is, aliquots of the membrane-filtered red wine sample (10 μL) were analyzed by HPLC-MS-MS in the negative ionization mode (ESI$^-$). By means of multiple reaction monitoring (MRM), individual flavonol glycosides were analyzed using the following transition reactions given in parentheses: isorhamnetin-3-*O*-β-D-glucopyranoside (*m/z* 477.1 → 313.9; DP, −115 V; EP, −10 V; CEP, −42 V; CE, −38 V; CXP, −17 V); quercetin-3-*O*-β-D-galactopyranoside (*m/z* 463.1 → 299.9; DP,−95 V; EP, −10 V; CEP, −42 V; CE, −38 V; CXP, −17 V); and syringetin-3-*O*-β-D-glucopyranoside (*m/z* 507.1 → 343.9; DP, −105 V; EP, −10 V; CEP, −44 V; CE, −40 V; CXP, −7 V).

Phenolic Acids, Phenolic Acid Ethyl Esters, and Furan-2-carboxylic Acid. Membrane-filtered aliquots of red wine (10 μL) were directly analyzed by means of HPLC-MS-MS as reported previously[10] using either positive ionization (ESI$^+$) for phenolic acids or negative ionization (ESI$^-$) for ethyl esters. Chromatography was performed on a 150 mm × 2.0 mm i.d., 5 μm, Synergi Fusion RP-18 column (Phenomenex Aschaffenburg, Germany) using the following gradient of acetonitrile containing 1% formic acid (solvent A) and 1% aqueous formic acid (solvent B) at a flow rate of 0.25 mL/min: increasing A from 0 to 35% within 20 min, then to 100% within 5 min, and, finally, isocratically with A for another 2 min. After identification of the individual compounds upon comparison of chromatographic (retention time) and spectroscopic data (LC-MS-MS) with those obtained for the reference compounds, quantitation was performed by comparing the peak area obtained for the trace of the corresponding mass transition with those of defined standard solutions of each reference compound in 20% aqueous methanol. Using the MRM mode, the taste compounds were analyzed using the following transition reactions: caffeic acid (*m/z* 181.1 → 89.0; DP, +21 V; EP, +6 V; CEP, +16 V; CE, +41 V; CXP, +4 V); gallic acid (*m/z* 171.1 → 108.9; DP, +31 V; EP, +12 V; CEP, +24 V; CE, +25 V; CXP, +2 V); *p*-coumaric acid (*m/z* 165.1 → 119.2; DP, +21 V; EP, +6 V; CEP, +14 V; CE, +25 V; CXP, +4 V); protocatechuic acid (*m/z* 155.1 → 123.2; DP, +41 V; EP, +12 V; CEP, +12 V; CE, +15 V; CXP, +4 V); syringic acid (*m/z* 199.2 → 140.2; DP, +31 V; EP, +9 V; CEP, +18 V; CE, +21 V; CXP, +4 V); vanillic acid (*m/z* 169.1 → 93.0; DP, +21 V; EP, +11 V; CEP, +14 V; CE, +19 V; CXP, +4 V); furan-2-carboxylic acid (*m/z* 113.0 → 69.1; DP, +31 V; EP, +7 V; CEP, +16 V; CE, +17 V; CXP, +4 V); protocatechuic acid ethyl ester (*m/z* 181.1 → 107.9; DP, −45 V; EP, −11 V; CEP, −16 V; CE, −30 V; CXP, −2 V); gallic acid ethyl ester (*m/z* 197.1 → 124.2; DP, −45 V; EP, −7 V; CEP, −18 V; CE, −30 V; CXP, −2 V); *p*-coumaric acid ethyl ester (*m/z* 191.1 → 116.8; DP, −40 V; EP, −7 V; CEP, −20 V; CE, −42 V; CXP, −0 V); caffeic acid ethyl ester (*m/z* 207.1 → 134.9; DP, −45 V; EP, −8 V; CEP, −16 V; CE, −30 V; CXP, −0 V).

Quantitative Analysis of Ellagitannins. Quantitation of ellagitannins, mainly castalagin and vescalagin, was performed as described recently.[19] Red wine was membrane-filtered, and aliquots (20 μL) were directly analyzed by HPLC-MS-MS. After identification of the individual compounds upon comparison of chromatographic and spectroscopic data with those obtained for the reference compounds, quantitation was performed by comparing the peak area obtained for the trace of the corresponding mass transition with those of defined standard solutions of each reference compound in a matrix red wine free of ellagitannins. Using MRM mode, vescalagin and castalagin (*m/z* 466.0 → 300.9; DP, −65 V; EP,−10 V; CE, −36 V; CXP, −11 V) were analyzed using the transition reaction given in parentheses.

Quantitative Analysis of Proline. L-Proline was quantified by means of stable isotope dilution assays following the method reported recently with slight modifications.[20] The concentration was calculated using a response curve determined by analysis of defined amounts of the labeled and unlabeled amino acid in different concentration ratios. Aliquots of red wine were diluted 1:1000 (v/v) with water and membrane-filtered, and aliquots (2 μL) were analyzed by HPLC-MS-MS using a 150 mm × 2 mm i.d., 3 μm, TSK-gel Amide 80 column (Tosoh Bioscience, Stuttgart, Germany). A 95% acetonitrile solution containing 5 mM ammonium acetate, adjusted to pH 3 with acetic acid, was used as solvent A, and 5 mM aqueous ammonium acetate buffer, adjusted to pH 3 with acetic acid, was used as solvent B. Separation was performed at a flow rate of 0.2 mL/min starting with an initial mixture of 85% solvent A and 15% solvent B for 3 min. Solvent B was then decreased within 7 min to 55%, then further decreased to 0% within 4 min, and, finally, kept at 0% for 4 min. Using MRM mode operating in the positive ionization mode (ESI$^+$), proline was analyzed after tuning the MS-MS parameters for each compound. The following mass transitions were used: L-proline (*m/z* 116.1 → 70.0; DP, +21 V; EP, +10 V; CE, +21 V; CXP, +4 V) and labeled L-proline ($^{13}C_{-5}$, ^{15}N) (*m/z* 122.0 → 75.0; DP, +73 V; EP, +10 V; CE, +25 V; CXP, +5 V).

Quantitative Analysis of Galacturonic Acid and (E)- and (Z)-Aconitic Acid. Red wine was diluted 1:10 and 1:50 (v/v) with water and membrane filtered, and aliquots (5 μL) were directly analyzed by means of LC-MS-MS in negative ionization mode (ESI$^-$) on a 150 mm × 4.6 mm i.d. ZIC-pHILIC column (Merck SeQuant, Umea, Sweden) using the following gradient of 5 mM aqueous ammonium acetate buffer in 95% acetonitrile, adjusted to pH 9 with ammonia (solvent A), and 5 mM aqueous ammonium acetate buffer pH 9 (solvent B) at a flow rate of 0.5 mL/min. Starting with a mixture of 80% solvent A and 20% solvent B for 5 min, the amount of solvent B was increased to 100% in 10 min and finally kept at 100% solvent B for a further 5 min. Using MRM mode, the compounds were analyzed using the following mass transitions, monitored for 10 ms: galacturonic acid (*m/z* 193.0 → 73.1; DP, −40 V; CEP, −18 V; CE, −18 V; CXP, −5 V); (*E*)- and (*Z*)-aconitic acid (*m/z* 173.1 → 128.8; DP, −10 V; CE, −10 V; CXP, −6 V). Quantitation was performed by external calibration on the basis of peak areas.

Quantitative Analysis of Organic Acids, Soluble Carbohydrates, Alditols, and Minerals by Means of High-Performance Ion Chromatography (HPIC). Quantitative analysis of organic acids, soluble carbohydrates, and alditols was performed by ion chromatography on a Dionex IC 2500 system (Dionex, Idstein, Germany) consisting of a GS50 gradient pump, an AS50 autosampler, an AS50 thermal compartment, and an ED 50 electrochemical detector. Cations were quantified using a Dionex ICS-2000 apparatus with a digital conductivity detector, a suppressor CSRS 300, an AS autosampler, and an eluent generator equipped with a RFIC EluGen cartridge EGC II MSA (Dionex). Data analysis was performed using Chromeleon software 6.80. For quantitation, external standard calibration was done in concentrations ranging from 0.5 to 100 mg/L (six-point calibration).

Carbohydrates and Alditols. Red wine was diluted 1:100 (v/v) with water for quantitation of carbohydrates and 1:10000 (v/v) for quantitation of glycerin, respectively. Aliquots (25 μL) were analyzed on a 250 mm × 4.0 mm i.d. Carbo Pac MA-1 column equipped with a 50 mm × 4.0 mm guard column of the same type and monitored with a pulsed amperometric detector equipped with a gold working electrode operating with a standard quadrupole waveform. The data collection rate was 2 Hz. Separation was performed at 30 °C at a flow rate of 0.4 mL/min using water and aqueous 1 M NaOH solution (52:48) for 70 min. By comparison of retention times with those of fructose, glucose, arabinose, galactose, rhamnose, and glycerin, the compounds were quantified using a six-point external standard calibration.

Organic Acids. Red wine (1.0 mL) was made up to 50 mL with water, and aliquots (25 μL) were analyzed on a 250 mm × 9.0 mm i.d. IonPac ICE-AS6 column (Dionex).[21] Acetic acid, lactic acid, tartaric acid, succinic acid, malic acid, and citric acid were quantified by means of a six-point external standard calibration.

Cations. Red wine was diluted 1:10 and 1:100 with water, and aliquots (10 μL) were analyzed on a 250 mm × 2.0 mm i.d. Ion-Pac CS-18 column (Dionex) equipped with a guard column (50 × 2.0 mm i.d.) of the same material at a temperature of 40 °C. Cations were eluted during 20 min using 5 mM methanesulfonic acid. Between the column and the conductivity detector, a cation self-regenerating suppressor, operating at 5 mA, was installed. The cations magnesium, potassium, ammonium, calcium, and sodium were quantified by means of a six-point external standard calibration.

Isolation of the High Molecular Weight (HMW) Fraction of Red Wine by Ultrafiltration. Following the protocol previously described,[10] the red wine sample (250 mL) was placed into a Vivacell 250 static gas pressure filtration system (Vivascience, Germany) equipped with a 5 kDa molecular weight cutoff Vivacell 250 5000 MWCO PES membrane and preconditioned by rinsing the membrane twice with deionized water (300 mL). After sealing, a pressure of 4 bar was applied using an air pressure controller, and the Vivacell 250 was placed on a type 3005 laboratory shaker (GFL, Germany) operating at 100 rpm and room temperature. After filtration, the retentate was taken up in 15% aqueous EtOH (100 mL) and filtered again. After this washing step had been repeated three times, the retentate was taken up in deionized water (50 mL) and the membrane was washed twice with 20% aqueous ethanol (20 mL) to remove adsorbed material. The combined materials were lyophilized to yield the HMW fraction (HMW > 5 kDa) in an amount of 3.3 g/L.

Sensory Analyses. *Panel Training.* To train the panel in recognizing and distinguishing different qualities of aroma and taste or aroma sensations in analytical sensory experiments, 18 persons participated in weekly training sessions. All panelists had given informed consent to participate in the sensory tests of the present investigation and had no history of known taste or smell disorders. For aroma evaluation, the subjects were trained to evaluate the odor of aqueous solutions (20 mL) of the following standard aroma compounds in water: flowery (2-phenylethanol, 42000 μg/L); malty (3-methyl-1-butanol, 66000 μg/L); fruity (ethyl 3-methylbutanoate, 6.9 μg/L); cooked apple-like ((*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one, 3.9 μg/L); clove-like (4-allyl-2-methoxyphenol, 1800 μg/L); sweaty (3-methylbutanoic acid, 150000 μg/L); smoky (2-methoxyphenol, 250 μg/L); vanilla-like (4-hydroxy-3-methoxybenzaldehyde, 16000 μg/L); coconut-like ((4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one, 2200 μg/L); vinegar-like (acetic acid, 30000000 μg/L); butter-like (2,3-butanedione, 300 μg/L); and cooked potato-like (3-(methylthio)propanal, 130 μg/L). For taste evaluation, the subjects were trained to evaluate the taste of aqueous solutions (2 mL) of the following taste compounds in bottled water (Evian, low mineralization = 500 mg/L) using the sip-and-spit method: sucrose (12.5 mmol/L) for sweet taste; caffeine (1 mmol/L) for bitter taste; NaCl (20 mmol/L) for salty taste; lactic acid (20 mmol/L) for sour taste; and monosodium L-glutamate (3 mmol/L) for umami taste. For the puckering astringency and the velvety astringent oral sensation, the panel was trained by using tannic acid (0.05%) and quercetin-3-*O*-β-D-glucopyranoside (0.01 mmol/L), respectively, using the half-tongue test.[22,23] For the training of mouthfullness/body, a red wine was spiked with increasing amounts of glycerol (5−20 g/L) and compared to the wine sample without additive. The sensory sessions were performed at 22 °C in an air-conditioned room.

Aroma and Taste Profile Analyses. This was performed by a trained sensory panel consisting of 18 panelists. The aroma and taste descriptors represented by the compounds used above for the training sessions were chosen for sensory evaluation of wine and the recombinate. Their intensities were ranked on a seven-point scale (steps of 0.5) from 0 (not perceivable) to 3 (strongly perceivable). The single judgments of the panelists were averaged.

Aroma and Taste Reconstitution Experiments. Model solutions containing all quantified aroma compounds and tastants at concentration levels equal to those determined in the red wine were prepared as given below. The samples (20 mL each) were placed in glass vessels (total volume = 45 mL). The model mixtures were presented to the panel at room temperature and judged in comparison to the red wine on a scale from 0 (not detectable) to 3 (strongly perceivable). The data are given as the mean of triplicates (relative standard deviation for each data point <±0.3 scale points).

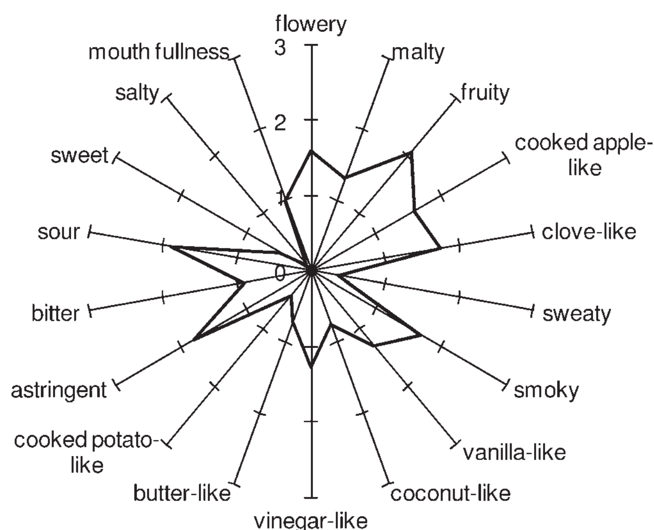

Figure 1. Aroma and taste profile analysis of Dornfelder red wine.

Studies on Taste/Aroma Interactions. Ethanolic solutions of odorants were dissolved in either an aqueous solution of ethanol (13% v/v) adjusted to pH 3.8 by hydrochloric acid (0.1 mol/L) (recombinate A; Rec A) or an aqueous solution of ethanol (13% v/v; pH 3.8) containing all low molecular weight tastants in the absence (recombinate B; Rec B) or presence (recombinate C; Rec C) of the HMW fraction (>5 kDa). Acetic acid was either added with the aroma compounds (Rec A) or was added with the taste compounds (Rec B and C). After equilibration for 2 h in the dark, the flavor qualities were evaluated by aroma and taste profile analysis as described above.

RESULTS AND DISCUSSION

To evaluate the flavor profile of the Dornfelder red wine on a scientific basis, first, aroma as well as taste profile analyses were performed on a seven-point scale (steps of 0.5) from 0 (not perceivable) to 3 (strongly perceivable) (Figure 1). Fruity was the aroma quality that was strongly perceivable by the panel, followed by clove-like, smoky, flowery, and cooked apple-like. The aroma quality sweaty was perceived by the panelists with the lowest intensity. Among the taste impressions, the highest intensities were detected for sour and astringent, which were evaluated with intensities of 1.9 and 1.8, respectively.

Because the key taste compounds were already identified in another red wine by means of a molecular sensory science approach,[9,10] first, the most important odor-active volatiles had to be identified prior to quantitative analysis and reconstitution of the typical flavor of the Dornfelder red wine.

Characterization of Aroma Compounds. Volatiles were isolated by solvent extraction of Dornfelder red wine, followed by high vacuum distillation. When a drop of the obtained distillate was sniffed on a strip of filter paper, the typical flowery, fruity, smoky aroma of the red wine was perceivable.

Application of GC-O to the distillate revealed 31 aroma-active areas, among which 3 compounds with a malty aroma, a flowery

Table 2. Important Odor-Active Compounds (FD ≥ 32) Identified in a Distillate Prepared from the Dornfelder Red Wine

compd no.	aroma compd[b]	odor quality[c]	RI[a] on FFAP	RI[a] on DB-5	FD[d]
1	ethyl methylpropanoate	fruity	968	754	1024
2	2,3-butanedione	butter-like	989	591	512
3	ethyl butanoate	fruity	1039	801	512
4	(*S*)-ethyl 2-methylbutanoate	fruity	1054	848	4096
5	ethyl 3-methylbutanoate	fruity	1072	855	1024
6	(*S*)-2 and 3-methyl-1-butanol	malty	1213	735	≥8192
7	ethyl hexanoate	fruity	1237	998	64
8	2-isopropyl-3-methoxypyrazine[e]	pea-like, earthy	1430	1094	512
9	acetic acid	vinegar-like	1445	597	512
10	3-(methylthio)propanal	cooked potato-like	1456	905	1024
11	2-isobutyl-3-methoxypyrazine[e]	bell pepper-like, earthy	1518	1178	32
12	2-methylpropanoic acid	sweaty	1565	762	128
13	butanoic acid	sweaty	1627	793	1024
14	(*S*)-2- and 3-methylbutanoic acid	sweaty, rancid	1666	846	4096
15	3-(methylthio)propan-1-ol	cooked potato-like	1714	981	1024
16	pentanoic acid	sweaty	1734	895	32
17	2-phenylethyl acetate	flowery	1809	1259	2048
18	(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one	cooked apple-like	1809	1383	2048
19	2-methoxpyhenol	smoky	1864	1089	1024
20	2-phenylethanol	flowery	1916	1116	≥8192
21	(4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one	coconut-like	1954	1327	512
22	4-ethyl-2-methoxyphenol	clove-like	2026	1279	256
23	5-pentyldihydrofuran-2-(3*H*)-one	coconut-like	2026	1367	256
24	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	caramel-like	2042	1060	128
25	2-ethyl-4-hydroxy-5-methylfuran-3(2*H*)-one[e]	caramel-like	2082	1145	32
26	4-allyl-2-methoxyphenol	clove like	2167	1356	1024
27	4-ethylphenol	phenolic	2182	1169	32
28	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like	2211	1106	≥8192
29	decanoic acid	fatty, musty	2273	1368	256
30	2-phenylacetic acid	honey-like	2570	1248	512
31	4-hydroxy-4-methoxybenzaldehyde	vanilla-like	2575	1404	2048

[a] Retention index. [b] The compound was identified by comparing its mass spectra (MS-EI, MS-CI) retention indices on capillaries FFAP and DB-5 as well as the odor quality and the odor intensity perceived during sniffing with data of reference compounds. [c] Odor quality perceived at the sniffing-port. [d] Flavor dilution factor determined by AEDA on capillary FFAP. [e] No unequivocal mass spectrum was obtained. Identification is based on the remaining criteria given in footnote "a".

note, and a seasoning-like odor were most intense. These compounds (**6**, **20**, and **28**) (Table 2) also showed the highest FD factors after ranking by application of the AEDA.

To identify the compounds responsible for the perceived odors, first, the retention indices of the odor-active areas were determined on two different stationary GC phases. A comparison with data of ∼1000 food odorants available in an in-house database proposed structures of each odorant. Then, the distillate was fractionated on silica gel,[16] in the single fractions obtained, the odorants were again located by GC-O, and their mass spectra were recorded. The data obtained were first cross-checked against the database, but the structure was finally confirmed by comparing the analytical and sensory attributes with those of the respective reference compounds. This procedure was necessary, because several trace odorants coeluted with odorless volatiles present in high amounts. Thus, without fractionation, the substances would have been incorrectly identified. Following this procedure, all 31 odorants detectable by GC-O could be identified.

The three most odor-active odorants in Dornfelder red wine were characterized as (*S*)-2- and 3-methyl-1-butanol (**6**), 2-phenylethanol (**20**), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**28**) (Table 2). With somewhat lower FD factors, (*S*)-ethyl 2-methylbutanoate (**4**, FD 4096) and 2-phenylethyl acetate (**17**, FD 2048) were identified as further key odorants (Table 2). Additionally, the sweaty, rancid (*S*)-2- and 3-methylbutanoic acid (**14**, FD 4096), the cooked apple-like (*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one (β-damascenone) (**18**, FD 2048), and the vanilla odor-like smelling 4-hydroxy-3-methoxybenzaldehyde (**31**, FD 2048) were suggested as potential contributors to the overall aroma of Dornfelder red wine by their high FD factors. Altogether, 33 odorants could be identified (Table 2). Analysis of the chiral constituents on two different chiral stationary phases revealed that ethyl 2-methylbutanoate, 2-methyl-1-butanol, and 2-methylbutanoic acid all occurred as their (*S*)-isomers in red wine. These findings are in agreement with data of previous studies on other wines.[24,25]

Table 3. Concentrations of 28 Important Aroma Compounds in Dornfelder Red Wine (Listed in Decreasing Concentration)

aroma compd	concn[a] (μg/L)	range (μg/L)
acetic acid	641900[b]	575200−721700
3-methyl-1-butanol	307200	306100−308400
2-phenylethanol	79040	78910−79170
(*S*)-2-methyl-1-butanol	77680	77380−77980
acetaldehyde	12100	12040−12160
2,3-butanedione	2040	1980−2110
3-methylbutanoic acid	1740[c]	1700−1790
2-methylpropanoic acid	1690	1520−1850
butanoic acid	1380[b]	1190−1470
(*S*)-2-methylbutanoic acid	957[c]	938−988
decanoic acid	476	449−503
ethyl methylpropanoate	385[b]	364−408
ethyl hexanoate	307	305−309
ethyl butanoate	239	232−246
4-hydroxy-3-methoxy-benzaldehyde	167	161−172
(4*S*,5*S*)-5-butyl-4-methyldihydrofuran-2(3*H*)-one	164	164−164
2-phenylacetic acid	102	101−103
ethyl 3-methylbutanoate	55.1	54.8−55.3
2-phenylethyl acetate	53.5	51.4−55.5
(*S*)-ethyl 2-methylbutanoate	50.6	48.6−52.6
4-ethyl-2-methoxyphenol	39.4	39.3−39.5
2-methoxyphenol	19.2	19.1−19.3
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	19.2	16.6−21.7
5-pentyldihydrofuran-2(3*H*)-one	10.7	10.5−10.9
4-allyl-2-methoxyphenol	7.3	7.2−7.5
3-(methylthio)propanal	3.3	3.2−3.4
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	3.2	3.2−3.2
(*E*)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)but-2-en-1-one	0.9	0.9−0.9

[a] Calculated as the mean value of two different workups (see range). [b] Calculated as the mean value of quadruplicates (see range). [c] Calculated as the mean value of triplicates (see range).

Quantitation of Aroma Compounds. A total of 27 compounds, judged with FD factors of ≥64 (Table 2), were quantitated by means of stable isotope dilution assays. In addition, acetaldehyde was quantitatively determined by means of an enzymatic assay. The highest concentrations were determined for acetic acid and 3-methyl-1-butanol followed by 2-phenylethanol (Table 3). In particular, the two alcohols are known constituents of yeast metabolism and are, therefore, volatile constituents of all alcoholic beverages. They are formed either by a degradation of the respective amino acids leucine and 2-phenylalanine, respectively, following the Ehrlich pathway, or by a side reaction in amino acid biosynthesis.

A total of 14 compounds were present in concentrations above 200 μg/L, but very low concentrations (below 5 μg/L) were found for 3 odorants, namely, 3-(methylthio)propanal, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, and β-damascenone.

Ferreira et al.[26] also reported on acetic acid as the odorant with the highest concentration in another red wine varietal followed by 3-methyl-1-butanol and 2-phenylethanol. Because nearly all aroma compounds identified in the Dornfelder red wine have been reported as odor-active constituents in red wines before, our data clearly support the idea that the different aroma profiles of red wine are obviously caused by quantitative rather than qualitative differences in the key odorants.

Table 4. Concentrations and Taste Qualities of Important Taste-Active Compounds in Dornfelder Red Wine (Listed in Decreasing Concentration)

taste compd	taste quality	concn[a] (mg/L)	range (mg/L)
glycerol	sweet	11972	11472−12471
high molecular weight fraction	astringent	3300	2900−3700
lactic acid	sour	2892[b]	2870−2913
fructose	sweet	2838	2720−2956
tartaric acid	sour	1765[b]	1729−1801
glucose	sweet	1162	1109−1215
potassium	salty, bitter	1056	1047−1066
galacturonic acid	sour	807.2	752.6−849.4
L-proline	sweet	601.5	530.0−673.1
succinic acid	sour	596.3[b]	590.7−601.9
acetic acid	sour	333.1[b]	318.9−347.3
magnesium	salty	62.7	52.3−73.0
calcium	salty	53.5	50.1−56.9
malic acid	sour	50.6[b]	48.9−52.3
sodium	salty	42.5	41.4−43.6
(*E*)-caftaric acid	astringent	38.9	37.4−40.5
citric acid	sour	23.7[b]	23.3−24.1
gallic acid	astringent	19.2	17.5−20.2
syringic acid	astringent	6.7	5.6−7.7
caffeic acid	astringent	5.5	5.0−6.0
(+)-catechin	astringent, bitter	4.9	4.7−5.0
ammonium	salty	4.5	4.3−4.6
gallic acid ethyl ester	astringent, bitter	4.4	4.1−4.5
vanillic acid	astringent	4.4	3.8−4.9
p-coumaric acid	astringent	4.2	4.1−4.3
(−)-epicatechin	astringent, bitter	4.1	4.0−4.1
protocatechuic acid ethyl ester	astringent, bitter	3.7	3.3−3.9
furan-2-carboxylic acid	astringent	3.2	3.2−3.3
syringetin-3-*O*-β-D-glucoside	velvety astringent	2.6	2.5−2.7
p-coumaric acid ethyl ester	astringent, bitter	1.5	1.4−1.7
(*Z*)-aconitic acid	sour, astringent	1.3	1.1−1.5
quercetin-3-*O*-β-D-galactoside	velvety astringent	1.3	1.2−1.5
castalagin	astringent	1.1	1.1−1.2
caffeic acid ethyl ester	astringent, bitter	0.8	0.8−0.9
(*E*)-aconitic acid	sour, astringent	0.4	0.3−0.4
isorhamnetin-3-*O*-β-D-glucoside	velvety astringent	0.2	0.2−0.2

[a] Calculated as the mean value of triplicates (see range). [b] Calculated as the mean value out of duplicates (see range).

Quantitation of Taste Compounds. Among the 82 taste-active compounds recently identified in an Amarone red wine,[9,10] it was shown that only 37 were necessary to reconstruct the overall taste impression in reconstitution experiments. On the basis of the data of this former study, 3 flavon-3-ol glucosides,

Table 5. Aroma and Taste Profile Analysis of Red Wine and Aroma and Aroma/Taste Recombinates

quality	intensities[a] in			
	red wine	Rec C[d]	Rec B[c]	Rec A[b]
aroma				
flowery	1.6	1.6	1.5	1.4
malty	1.3	1.5	1.4	1.7
fruity	2.1	2.1	2.4	2.3
cooked apple-like	1.6	1.7	1.3	1.2
clove-like	1.8	1.8	1.6	1.2
sweaty	0.4	0.4	0.6	0.8
smoky	1.7	1.9	1.4	1.1
vanilla-like	1.3	1.4	1.3	1.0
coconut-like	0.8	1.0	0.9	0.9
vinegar-like	1.3	1.4	1.6	1.4
butter-like	0.7	0.9	0.9	0.8
cooked potato-like	0.4	0.6	0.4	0.6
taste				
astringent	1.8	1.9	1.3	ne[e]
bitter	0.9	0.8	0.8	ne
sour	1.9	1.7	1.9	ne
sweet	0.5	0.6	0.7	ne
salty	0.1	0.1	0.1	ne
mouthfullness	1.0	1.1	0.9	ne

[a] Intensities were rated on a seven-point scale from 0 (not detectable) to 3 (strong impression). [b] The cocktail of odorants, each in its natural concentration (Table 3), was dissolved in an aqueous solution of ethanol (13% v/v; pH 3.8). [c] The cocktail of odorants (Table 3) and the low molecular weight taste compounds, each in natural concentration (Table 4), were dissolved in an aqueous solution of ethanol (13% v/v; pH 3.8). [d] The cocktail of odorants (Table 3) and the low molecular weight taste compounds as well as the high molecular weight fraction (>5 kDa), each in natural concentration (Table 4), was dissolved in an aqueous solution of ethanol (13% v/v; pH 3.8). [e] ne, not evaluated.

10 phenolic acid derivatives, 2 flavan-3-ols, 1 amino acid, 2 carbohydrates, 1 alditol, 9 organic acids, furan-2-carboxylic acid, 5 cations, and 2 ellagitannins, as well as the HMW fraction (>5 kDa), were quantitatively determined in the Dornfelder red wine (Table 4).

Among the puckering astringent components, the HMW fraction was present in by far the highest concentration of 3.3 g/L, followed by (*E*)-caftaric acid, gallic acid, syringic acid, caffeic acid, vanillic acid, *p*-coumaric acid, and furan-2-carboxylic acid, with concentrations between 38.9 and 3.2 mg/L, and (*Z*)- and (*E*)-aconitic acid and the ellagitannin castalagin, with concentrations between 1.3 and 0.4 mg/L (Table 4). Among the ellagitannins, which are known to be extracted from the wood during aging, only castalagin was present in high concentration, as already shown for several different red wines.[19] Besides the puckering astringent compounds, the Dornfelder wine was screened for velvety astringent flavon-3-ol glycosides. The highest concentration of 2.6 mg/L was found for syringetin-3-*O*-*β*-D-glucopyranoside, followed by quercetin-3-*O*-*β*-D-galactopyranoside and isorhamnetin-3-*O*-*β*-D-glucopyranoside with concentrations of 1.3 and 0.2 mg/L (Table 4).

As representatives of the astringent and bitter-tasting molecules, phenolic acid ethyl esters and flavan-3-ols were quantified in the red wine (Table 4). The flavan-3-ols (+)-catechin and (−)-epicatechin as well as the esters gallic acid ethyl ester and protocatechuic acid ethyl ester were present in concentrations between 4.9 and 3.7 mg/L, followed by *p*-coumaric ethyl ester and caffeic ethyl ester with somewhat lower amounts of 1.5 and 0.8 mg/L, respectively.

Finally, compounds exhibiting a sour or salty taste were quantified. Lactic acid was the predominant organic acid with a concentration of 2.9 g/L, followed by tartaric acid (1.8 g/L), galacturonic acid (807 mg/L), succinic acid (596.3 mg/L), and acetic acid (333.1 mg/L) (Table 4). Among the minerals, potassium reached a concentration of 1.0 g/L and, besides saltiness, it is known to exhibit a bitter taste impression in higher concentrations.[10]

Aroma and Taste Reconstitution Experiments. Because it is difficult to predict the overall aroma or taste of a complex mixture of compounds, flavor reconstitution experiments are a useful tool to validate the correctness of the quantitative data. To verify the identification experiments, a recombination experiment was performed using the cocktail of all aroma and taste compounds, each in the concentration given in Tables 3 and 4, in 13% ethanolic solution (Rec C). The aroma and taste profiles of the authentic red wine and that of recombinate were in good agreement (Table 5). In the wine and also in the recombinate, the fruity aroma quality was perceived with the highest score by the panelists. In addition, all aroma descriptions were evaluated to be nearly similar in the wine and the recombinate; for example, flowery, fruity, clove-like, and sweaty were judged to have the same intensity. Also, all six taste qualities were ranked similarly in the recombinate and the wine and, especially, the values of the attributes astringency, bitterness, and mouthfullness matched very well. These data confirmed that the key aroma and taste compounds of Dornfelder red wine were successfully identified and quantified.

In a second set of experiments, all aroma compounds were dissolved in 13% ethanol without the addition of tastants (Rec A). In comparison to Rec C, containing all tastants, in particular, the smoky, clove-like, cooked apple-like, and vanilla-like quality was lower, whereas the sweaty note was higher in Rec A. That is, the smoky note was judged with an intensity of 1.9 in Rec C compared to 1.1 in Rec A, clove-like had an intensity of 1.8 in Rec C and 1.2 in Rec A, cooked apple-like was evaluated with 1.7 in Rec C and with 1.2 in Rec A, and also for vanilla-like there was a clear difference. To confirm that the differences in the aroma profiles were not caused by odorants possibly present in the fraction of nonvolatiles, a solution of all taste compounds (Table 4) in 13% ethanol was orthonasally evaluated. Because no odor was detected in a triangle test using pure 13% ethanol as the blank (data not shown), it can be concluded that the nonvolatile compounds themselves had a clear influence on the release of the aroma compounds from the matrix.

In a third experiment, the entire mix of odorants was added to a solution of tastants, but the astringent HMW fraction was omitted (Rec B) (Table 5). As to be expected, Rec B lacking the HMW fraction showed a significantly reduced astringent sensation (Table 5), thus demonstrating the key role of these polymers in the astringent perception of the red wine. However, the HMW fraction also seemed to influence the perception of the other taste qualities, because, for example, Rec B showed a lower intensity for mouthfullness compared to recombinate C (Table 5) and also a somewhat higher intensity of sourness. Vidal et al.[27] have previously demonstrated that polysaccharides had an influence on the perceived fullness of a model wine solution. The slight increase of mouthfullness in Rec C compared to Rec B might, therefore, be attributed to the addition of polysaccharides present in the HMW fraction.

A change of the aroma profile due to interactions with the nonvolatile matrix was also described previously in other studies.[11−15] By combining volatile and nonvolatile extracts isolated from white and red wines Sáenz-Navajas et al.[15] found that differences were particularly notable for esters and acids. Also, in the presence of catechin a decrease of volatility of isoamyl acetate and ethyl hexanoate was previously observed.[12] The slight decrease of the fruity aroma quality in Rec C in comparison to Rec A could, thus, be explained by a possible interaction of esters with the matrix. In contrast to phenolic compounds, wine polysaccharides, in a concentration range typical for wine, were not to found have a significant effect on the aroma of wine.[28] However, to get detailed insights into such interactions, further studies must be performed on the molecular level.

ASSOCIATED CONTENT

Supporting Information. Preparation of isotopically labeled compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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