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Quantitative Reconstruction of the Nonvolatile Sensometabolome of a Red Wine

JAN CARLOS HUFNAGEL[†] AND THOMAS HOFMANN*[,#]

Institut für Lebensmittelchemie, Universität Münster, Corrensstrasse 45, D-48149 Münster, Germany, and Lehrstuhl für Lebensmittelchemie and Molekulare Sensorik, Technische Universität München, Lise-Meitner-Strasse 34, D-85354 Freising-Weihenstephan, Germany

The first comprehensive quantitative determination of 82 putative taste-active metabolites and mineral salts, the ranking of these compounds in their sensory impact based on dose-over-threshold (DoT) factors, followed by the confirmation of their sensory relevance by taste reconstruction and omission experiments enabled the decoding of the nonvolatile sensometabolome of a red wine. For the first time, the bitterness of the red wine could be demonstrated to be induced by subthreshold concentrations of phenolic acid ethyl esters and flavan-3-ols. Whereas the velvety astringent onset was imparted by three flavon-3-ol glucosides and dihydroflavon-3-ol rhamnosides, the puckering astringent offset was caused by a polymeric fraction exhibiting molecular masses above >5 kDa and was found to be amplified by the organic acids. 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, respectively. In addition, D-fructose and glycerol as well as subthreshold concentrations of glucose, 1,2-propandiol, and *myo*-inositol were found to be responsible for the sweetness, whereas the mouthfulness and body of the red wine were induced only by glycerol, 1,2-propandiol, and *myo*-inositol.

KEYWORDS: Taste; red wine; astringency; bitterness; mouthfulness; taste reconstruction; sensometabolome

INTRODUCTION

It has been known for a long time that the typical taste profile of red wine centers around the orosensory qualities sourness, sweetness, bitterness, and astringency, respectively. Whereas velvety astringency is perceived as a silky and finely textured kind of astringent sensation, puckering astringency is understood as a reflexive action of cheek surfaces being brought together and released in an attempt to lubricate mouth surfaces (*1*). In addition, terms such as "mouthfulness", "body", and "complexity" are often used by wine experts to fully describe the oral sensation perceived during wine consumption.

It is well accepted that the comprehensive population of sensory active, low molecular weight compounds, coined "sensometabolome" (*2*), reflects the sensory phenotype and triggers the typical smell and taste of food products. The goal of our sensometabolomics program is therefore to catalog, quantify, and evaluate the sensory activity of metabolites that are present in raw materials and/or are produced upon food processing and storage, respectively (*3*). Despite the sensory importance of nonvolatiles in red wines, knowledge of the key metabolites inducing the typical orosensory profile and, in particular, the astringency and bitterness as well as mouthfulness/body of a red wine is still far from comprehensive.

Although many attempts have been made to correlate analytical data on distinct wine components with the sensory data obtained from human subjects, the reports of the chemical species imparting the typical taste profile of red wines are rather contradictory. For example, flavan-3-ols and proanthocyanidins were reported to be able to induce oral astringency as well as bitterness (*4*−*10*) and galloylated tannin polymers were found to induce a puckering astringent mouthfeel (*11*). In contradiction, studies performed on crude fractions isolated from red wines (*12*−*14*) revealed that gallic acid and, in particular, flavan-3-ols exhibiting molecular masses below 500 Da elicit astringent and bitter taste qualities. In addition, some organic acids were reported to impart astringency (*15*), whereas other studies found that organic acids are able to influence but, with the exception of malic acid, do not to evoke astringent taste sensations (*16, 17*). These findings clearly indicate that the key inducers of astringency are not yet unequivocally identified on a molecular level (*18*).

Similar to astringency, also the data available on bitter taste compounds in wines are rather inconsistent. Multiple investigations suggest procyanidins as bitter stimuli in wines (*4, 11, 12, 19, 20*), and ethanol was reported to enhance the bitter intensity

* Author to whom correspondence should be addressed (telephone +49-8161/71-2902; fax +49-8161/71-2949; e-mail thomas.hofmann@wzw.tum.de).

[†] Universität Münster.

[#] Technische Universität München.

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perceived (*5, 19*). In addition, terpene glycosides were reported to contribute to the bitter taste of Muscat wines (*21*). However, studies aimed at correlating chemical and sensory data of red wines were not successful in generating a predictive model for bitterness in wines (*22*).

To molecularize the key players driving the attractive taste of a red wine, we recently applied the so-called taste dilution analysis (TDA) to a red wine, namely, an Amarone della Valpollicella, to identify the key compounds exhibiting velvety astringency and puckering astringency, as well as bitterness (*23*). In this study, a total of 26 orosensory-active nonvolatiles were identified, among which several hydroxybenzoic acids, hydroxycinnamic acids, flavon-3-ol glycosides, and dihydroflavon-3-ol rhamnosides as well as a polymeric fraction (>5 kDa) were found to be the important astringent compounds, and several flavan-3-ols as well as a series of hydroxybenzoic acid ethyl esters and hydroxycinnamic acid ethyl esters were found as bitter compounds in the red wine (*23*).

To the best of our knowledge, no comprehensive quantitative study on the entire sensometabolome of a red wine has been performed so far. The objective of this work was to catalog and quantify putative taste-active metabolites, to rank them in their sensory impact based on dose/activity considerations, and to prove their sensory relevance by re-engineering the nonvolatile sensometabolome of the red wine.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: vanillic acid ethyl ester, caftaric acid (Apin Chemicals, Oxon, U.K.); *p*-coumaric acid, vanillic acid, gallic acid ethyl ester, mineral salts (Fluka Chemika, Taufkirchen, Germany); isorhamnetin-3-*O*-β-D-glucopyranoside, quercetin-3-*O*-β-D-galactopyranoside, syringetin-3-*O*-β-D-glucopyranoside, procyanidin B1, procyanidin B2 (Extrasynthese, Genay Cedex, France); alditols (Aldrich, Steinheim, Germany); amino acids (Merck, Darmstadt, Germany); all other chemicals were purchased from Sigma (Steinheim, Germany); solvents were of HPLC grade (Merck). Reference materials of procyanidins B3 and C1 were isolated from cocoa beans (*24*); quercetin-3-*O*-β-D-glucuronopyranoside, dihydroquercetin-3-*O*-α-L-rhamnoside, dihydrokaempferol-3-*O*-α-L-rhamnoside were isolated from red grapes (*23*); and ethyl esters of *p*-coumaric acid, caffeic acid, and syringic acid were synthesized as reported recently (*23*). The red wine, Amarone della Valpolicella DOC, used for the study was identical to that previously reported (*23*).

Quantitative Analysis of Flavan-3-ols. For the quantification of (+)-catechin, (−)-epicatechin, and oligomeric procyanidins, the red wine was diluted 1:1 with 0.1% aqueous formic acid and, after membrane filtration, aliquots (5 μL) were analyzed by means of LC-MS/MS in negative mode on an RP-18 Synergi Fusion, 150 × 2.0 mm i.d., 5 μm column (Phenomenex, Aschaffenburg, Germany) using the following gradient of methanol containing 0.1% formic acid (solvent A) and 0.1% aqueous formic acid (solvent B) at a flow rate of 0.2 mL/min: chromatography was performed starting with 5% solvent A for 5 min, then increasing the content of solvent A to 40% within 45 min, then to 100% within 5 min, and, finally, holding at 100% solvent A for another 10 min. After identification of the individual flavan-3-ols upon comparison of chromatographic (retention time) and spectroscopic data (LC-MS/MS, UV−vis) with those obtained for the reference compounds, quantification 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 methanol. By means of the multiple reaction monitoring (MRM) mode operated in the negative ionization mode, the individual flavan-3-ols were analyzed using the following transition reactions given in parentheses: (−)-epicatechin (*m/z* 289.1 → 245.0), (+)-catechin (*m/z* 289.1 → 245.0), (−)-epicatechin-(4β→8)-(+)-catechin (procyanidin B1; *m/z* 577.2 → 289.0), (−)-epicatechin-(4β→8)-(−)-epicatechin (procyanidin B2; *m/z* 577.2 → 289.0), (+)-catechin-(4α→8)-(+)-catechin (procyanidin B3; *m/z* 577.2 → 289.0), and (−)-epicatechin-(4β→8)$_2$-(−)-epicatechin (procyanidin C1; *m/z* 865.3 → 125.0).

Quantitative Analysis of (E)-Caftaric Acid, Flavon-3-ol, and Dihydroflavon-3-ol Glycosides. A sample of the red wine was 1:1 diluted with 0.1% aqueous formic acid and membrane filtered, and aliquots (5 μL) were analyzed by means of HPLC-MS/MS operated in the negative ionization mode on a Zorbax Eclipse XDB-C8, 150 × 2.1 i.d., 5 μm column (Agilent) using the following mass transitions: caftaric acid (*m/z* 311.1 → 177.7), isorhamnetin-3-*O*-β-D-glucopyranoside (*m/z* 477.2 → 313.9), quercetin-3-*O*-β-D-galactopyranoside (*m/z* 463.1 → 300.0), syringetin-3-*O*-β-D-glucopyranoside (*m/z* 507.1 → 343.9), quercetin-3-*O*-β-D-glucuropyranoside (*m/z* 477.1 → 300.9), dihydroquercitin-3-*O*-α-L-rhamnoside (*m/z* 449.1 → 150.8), dihydrokaempferol-3-*O*-α-L-rhamnoside (*m/z* 433.2 → 151.9). Chromatography was performed using the following solvent gradient at a flow rate of 0.25 mL/min: starting with a mixture (17:83, v/v) of acetonitrile, containing 0.1% formic acid, and 0.1% aqueous formic acid for 15 min, the acetonitrile content was increased to 30% within 30 min, then increased to 100% within 5 min, and, finally, held at 100% for an additional 10 min. Quantitative analysis was performed by comparing the peak areas obtained for the corresponding mass traces with those of standard solutions of each reference compound in a mixture (1:1, v/v) of methanol and 0.1% aqueous formic acid solution.

Quantitative Analysis of Polyphenolic Acids and Esters as well as Furan-2-carboxylic Acid. A sample of the red wine was 1:1 diluted with 0.1% aqueous formic acid and membrane filtered, and aliquots (5 μL) were analyzed by means of LC-MS/MS in the positive ionization mode on an RP-18 Synergi Fusion, 150 × 2.0 mm i.d., 5 μm column (Phenomenex) using the following gradient of acetonitrile containing 0.1% formic acid (solvent A) and 0.1% aqueous formic acid (solvent B) at a flow rate of 0.25 mL/min: chromatography was performed by increasing the content of solvent A from 0 to 35% within 50 min, then increasing the content of solvent A to 100% within 5 min, and, finally, holding at 100% solvent A for another 10 min. After identification of the individual compounds upon comparison of chromatographic (retention time) and spectroscopic data (LC-MS/MS, UV−vis) with those obtained for the reference compounds, quantification 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 mixture (1:1, v/v) of acetonitrile and 0.1% aqueous formic acid. Using the MRM mode, the taste compounds were analyzed using the following transition reactions: caffeic acid (*m/z* 181.1 → 163.0), gallic acid (*m/z* 171.1 → 153.1), *p*-coumaric acid (*m/z* 165.1 → 147.0), ferulic acid (*m/z* 195.1 → 176.9), protocatechuic acid (*m/z* 155.1 → 93.0), syringic acid (*m/z* 199.1 → 139.9), vanillic acid (*m/z* 169.0 → 93.1), furan-2-carbocylic acid (*m/z* 112.9 → 95.0), ferulic acid ethyl ester (*m/z* 223.2 → 177.2), vanillic acid ethyl ester (*m/z* 197.1 → 93.1), protocatechuic acid ethyl ester (*m/z* 183.1 → 155.1), gallic acid methyl ester (*m/z* 185.3 → 153.0), gallic acid ethyl ester (*m/z* 199.2 → 127.1), *p*-coumaric acid ethyl ester (*m/z* 193.1 → 147.1), syringic acid ethyl ester (*m/z* 226.8 → 140.0), caffeic acid ethyl ester (*m/z* 209.4 → 163.0).

Quantitative Analyses of Organic Acids, Amino Acids, Soluble Carbohydrates, Alditols, and Minerals. Quantitative analysis of organic acids, amino acids, soluble carbohydrates, and minerals was performed by means of an ICS 2500 ion chromatography system (Dionex, Idstein, Germany) equipped with an AS 50 thermal compartment, a GS 50 gradient pump, an ED 50 electrochemical detector, an AS 50 A autosampler, and a GM-4 gradient mixer as detailed previously (*3*). Analysis of data was performed with Chromeleon software v 6.60 SP4.

Carbohydrates. A sample of red wine (1.0 mL) was made up to 100 mL with water, and an aliquot (5 μL) was analyzed on a Carbo Pac PA-10, 250 × 2.0 mm i.d., column equipped with a 50 × 2.0 mm guard column of the same type and detected by the ED50-type pulsed amperometric detector. Chromatography was performed at 30 °C at a flow rate of 0.25 mL/min using the following gradient of water (eluent A) and an aqueous 250 mmol/L NaOH solution (eluent B): A/B (91:9, v/v) for 20 min, then to A/B (0:100, v/v) within 20 min and, finally, held isocratically for 10 min. By comparison of retention times and cochromatography with reference compounds of fructose, glucose,

arabinose, galactose, rhamnose, and xylose, these soluble carbohydrates were quantified using a six-point standard calibration.

Alditols. A sample of red wine (1.0 mL) was made up to 100 mL with water, and an aliquot (5 μL) was analyzed on a CarboPac MA-1, 250 × 2.0 mm i.d., column equipped with a CarboPac MA-1, 50 × 2.0 mm i.d., guard column and detected by the ED50-type pulsed amperometric detector. The identity of single compounds was verified by comparison of retention times and cochromatography with the following alditols: glycerol, 1,2-propanediol, inositol, mannitol, arabitol, erythritol, sorbitol, ribitol, and 2,3-butanediol. The concentration of each alditol in red wine was determined by comparison of the peak areas obtained for the individual compounds with those of defined standard solutions of each reference compound in deionized water.

Organic and Inorganic Acids. A sample of red wine (1.0 mL) was made up to 20 mL with water, and an aliquot (5 μL) was analyzed on an Ion Pac AS 11-HC, 250 × 2.0 mm i.d., column connected with an Ion Pac AG 11-HC, 50 × 2.0 mm, guard column and an anion self-regenerating suppressor ASRS Ultra II 2.0 mm, current 76 mA (Dionex), installed between the column and the ED50-type conductivity detector. Anions of the following organic and inorganic acids were quantified using a six-point standard calibration: formic acid, acetic acid, lactic acid, (*E*)-aconitic acid, (*Z*)-aconitic acid, glutaric acid, tartaric acid, succinic acid, malic acid, citric acid, isocitric acid, galacturonic acid, hydrochloric acid, and phosphoric acid, respectively.

Cations. A sample of red wine (1.0 mL) was made up to 10 mL with water, and an aliquot (10 μL) was analyzed on an Ion Pac CS 16, 250 × 3.0 mm i.d., column equipped with an Ion Pac CG 16, 50 × 3.0 mm i.d., guard column and a cation self-regenerating suppressor CSRS Ultra II 2.0 mm, current 37 mA (Dionex), installed between the column and the conductivity detector. The cations of magnesium, potassium, ammonium, calcium, and sodium were quantitatively analyzed by means of a six-point standard calibration.

Amino Acids. An aliquot (5 mL) of red wine was spiked with 0.5 mL of an aqueous solution of L-norleucine (2.5 mmol/L in 0.1 M HCl) as internal standard and applied onto the top of a Strata SCX cartridge (500 mg; Phenomenex, Torrance, CA) conditioned with 1 column volume of methanol, followed by 3 column volumes of deionized water. After the column had been flushed with 2 column volumes of deionized water, the cartridge was dried under vacuum for 5 min, and the amino acids were then eluted by flushing the cartridge twice with 5 mL of $CaCl_2$ solution (0.2 mol/L), followed by 5 mL of deionized water. The effluent was combined and made up to 50 mL with deionized water, and an aliquot (10 μL) of the solution was used for chromatography on an Amino Pac PA-10, 250 × 2.0 mm i.d., column equipped with an Amino Pac PA-10, 50 × 2.0 mm, guard column in combination with the ED50-type pulsed amperometric detector. By comparison of the retention times and cochromatography with reference compounds, L-arginine, L-lysine, L-glutamine, L-asparagine, L-alanine, L-threonine, glycine, L-valine, L-serine, L-proline, L-isoleucine, L-leucine, L-methionine, L-histidine, L-phenylalanine, L-glutamic acid, L-aspartic acid, and L-tyrosine were identified in wine and quantified via the internal standard L-norleucine.

Isolation of Red Wine Polymers by Means of Ultrafiltration. An aliquot (250 mL) of the red wine 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 nitrogen pressure of 4 bar was applied using an air pressure controller. During filtration Vivacell 250 was moved on a type 3005 GFL laboratory shaker at 200 rpm at room temperature. After filtration, the retentate was taken up with a 15% aqueous EtOH solution (100 mL) and again ultrafiltered under pressure (4 bar). 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 15% aqueous ethanol (20 mL) to remove adsorbed material. The combined materials were lyophilized to afford the high molecular weight fraction (HMW > 5 kDa) in a yield of 5.4 g/L. The fraction HMW > 5 kDa was kept at −18 °C until used for taste experiments.

Sensory Analyses. *General Conditions, Panel Training.* To familiarize the subjects with the taste language used by our sensory group and to get them trained in recognizing and distinguishing different qualities of oral sensations in analytical sensory experiments, 10 subjects (5 women and 5 men, ages 22−39 years), who had given informed consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated for at least two years in weekly training sessions. For example, the subjects were trained to evaluate the taste of aqueous 15% vol ethanolic solutions (2 mL; pH 3.8) of the following standard taste compounds in bottled water (Evian; low mineralization, 500 mg/L) using the sip-and-spit method: NaCl (20 mmol/L) for salty taste, lactose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, monosodium L-glutamate (3 mmol/L) for umami taste, and salicin (1.4 mmol/L) for bitter taste. For the puckering astringency and the velvety astringent oral sensation, the panel was trained by using gallustannic acid (0.05%) and quercetin-3-*O*-β-D-glucopyranoside (0.01 mmol/L), respectively, using the half-tongue test (*25, 26*). For the training of viscosity, an aqueous gelatin solution (0.5% in water) was used. For the training of mouthfulness/body, a red wine was spiked with increasing amounts of glycerol (5−20 g/L) and compared to the wine sample lacking any additive. The sensory sessions were performed at 22 °C in an air-conditioned room with separated booths in three independent sessions under red light. To prevent cross-modal interactions with odorants, the panelists used nose-clips.

Recognition Threshold Concentrations. Threshold concentrations of purified sour, sweet, bitter, salty, and umami tasting compounds were determined in bottled water adjusted to pH 3.8 with trace amounts of formic acid (1% in water) using triangle tests with ascending concentrations of the stimulus following the procedure reported previously (*26*). To overcome carry-over effects, astringent tasting compounds were evaluated by means of the recently developed half-tongue test (*25, 26*).

Taste Profile Analysis. A freshly opened bottle of wine was kept at room temperature for at least 2 h prior to sensory analysis. The samples (5 mL) were presented to the sensory panelists who wore nose-clips and were asked to briefly swirl the sample in the mouth and, then, to expectorate. Using this sip-and-spit method, the panelists were asked to score the taste qualities astringent, bitter, sour, sweet, salty, umami, and mouthfulness/body on a scale from 0 (not detectable) to 5.0 (strong taste impression).

Taste Reconstitution. To reconstitute the taste profile of the Amarone red wine, the "natural" concentrations of the taste compounds summarized in **Table 2** were dissolved in water, and the pH value of that solution was then adjusted to the initial pH value of the red wine (pH 3.8) by the addition of trace amounts of 0.1% aqueous formic acid. After an equilibration time of 15 min, the overall taste quality was evaluated by means of the taste profile analysis with the use of nose-clips. To avoid degradation of unstable polyphenols, exclusively freshly prepared solutions were tested.

Taste Omission Experiments. To evaluate the individual taste contribution of distinct taste compounds, partial taste recombinants were prepared by omitting either individual tastant groups or single taste compounds from the total taste recombinant. Each of the partial recombinants was presented to the panelists in comparison with the total taste recombinant using a triangle test. Panelists were asked to evaluate whether the solutions were identical in the overall taste or not. Those panelists who detected the odd sample correctly were asked to rate the intensity of the given taste descriptors of that sample on a scale from 0 (not detectable) to 5 (strongly detectable).

Liquid Chromatography−Mass Spectrometry (LC-MS/MS). Mass spectral analysis was performed in turbo spray electrospray ionization (ESI) mode on an API 4000 Q-Trap LC-MS/MS system (AB Sciex Instruments, Darmstadt, Germany), connected to an Agilent 1100 series HPLC system (Agilent, Karlsruhe, Germany). Samples were analyzed by loop injection (2−20 μL) at a flow rate of 200 μL/min (50:50, v/v, methanol/0.1% aqueous formic acid). The ion spray voltage was set at −4500 V in the ESI^- mode and at +5500 V in the ESI^+ mode, and the temperature was set to 300 °C. Nitrogen served as the curtain gas (20 psi), gas 1 (35 psi), and gas 2 (40 psi). The declustering potential was set at −10 to −30 V in the ESI^- mode and +60 V in the ESI^+ mode. The mass spectrometer was operated in the MRM mode, monitoring positive or negative ions. Fragmentation of $[M - H]^-$ and $[M + H]^+$ molecular ions into specific product ions was tuned by flow

Table 1. Taste Profile Analysis of the Authentic Amarone Red Wine and the Total Taste Recombinant Containing 82 Compounds

taste quality	intensities of individual taste qualities in[a]	
	red wine	total taste recombinant[b]
puckering astringent offset	4.0	4.1
velvety astringent onset	2.5	2.5
mouthfulness, body	4.0	3.8
bitter	1.5	1.3
sweet	2.0	1.9
sour	3.0	3.2
salty	0	0
umami	0	0

[a] Intensities were rated on a scale from 0 (not detectable) to 5 (strong taste impression). [b] The total taste recombinant consisted of the 82 taste compounds identified.

injection (10 μL/min) induced by collision with nitrogen (4×10^{-5} Torr). Analysis of mass spectrometry data was performed with Analyst software v 1.4.1.

RESULTS AND DISCUSSION

To evaluate the taste profile of the red wine on a scientific basis, a taste profile analysis using a five-point scale was performed (**Table 1**). The highest score of 4.0 was given for body/mouthfulness as well as for the puckering astringent offset, followed by sourness (3.0). The velvety mouthcoating onset, perceived by the sensory panel in the first part of the sensation, was judged with an intensity of 2.5, closely followed by sweetness (2.0). In comparison, bitterness was perceived with a lower intensity of 1.5, whereas umami and salty tastes were not detected at all.

Very recently, the orosensory-directed fractionation of the Amarone red wine (*23*) led to the identification of 26 sensory-active nonvolatiles among which several hydroxybenzoic acids and hydroxycinnamic acids as well as a structurally undefined polymeric fraction exhibiting molecular masses above 5 kDa were identified as puckering astringent components, whereas flavon-3-ol glucosides and dihydroflavon-3-ol rhamnosides exhibited a more velvety, silky-type of astringency. Besides some astringency, a series of hydroxybenzoic acid ethyl esters and hydroxycinnamic acid ethyl esters as well as procyanidins were evaluated as bitter stimuli in red wine.

The following experiments were performed to investigate the sensory contribution of the recently identified phenolic components (*23*) as well as organic acids, amino acids, soluble carbohydrates, alditols, and minerals to the taste of the red wine. To demonstrate a correlation between single-taste compounds and individual taste qualities, we aimed at re-engineering the entire nonvolatile sensometabolome of the wine by preparing a cocktail containing all of these putative taste compounds in their "natural" concentrations and to compare the taste profile of this biomimetic taste recombinant to that of the authentic wine. To achieve this, first, the entire set of basic taste compounds as well as the astringent and/or bitter phenolic constituents were quantified in the red wine, and the taste recognition thresholds were determined.

Concentrations and Dose-over-Threshold (DoT) Factors of Taste Compounds in Red Wine. From the putative taste compounds mentioned above, 4 flavon-3-ol glucosides, 2 dihydroflavon-3-ol glycosides, 17 phenolic acid derivates, 6 procyanidins, 18 amino acids, 13 carbohydrates and alditols, 13 organic acids, furan-2-carboxylic acid, and 7 cations and anions as well as the HMW polymers (>5 kDa) were quantitatively determined in the Amarone red wine. In addition, the taste recognition threshold concentrations of these compounds were determined, and a DoT factor was calculated for each compound from the ratio of the concentration and the threshold concentration (*27*). As we aimed to elucidate the key metabolites for each individual taste quality, the single-taste compounds were grouped into five classes differing in their taste qualities (**Table 2**).

Group I consisted of astringent but nonbitter tasting compounds, namely, four flavon-3-ol glucosides, two dihydroflavon-3-ol rhamnosides, nine phenolic acids, furan-2-carboxylic acid, and gallic acid methyl ester, as well as the high molecular weight polymers isolated from the red wine by means of ultrafiltration using a cutoff of 5 kDa (**Table 2**). Whereas the puckering astringent hydroxybenzoic acids exhibited rather high taste thresholds between 206 and 665 μmol/L, the series of hydroxycinnamic acids showed somewhat lower taste thresholds down to 16 μmol/L as found for (*E*)-caftaric acid. When compared to the puckering astringent phenolic acids, the flavon-3-ol-glucosides and dihydroflavon-3-ol-rhamnosides induced a more silky, velvety-like astringent oral sensation at drastically lower recognition thresholds; for example, syringetin-3-*O*-β-D-glucopyranoside exhibited a rather low threshold concentration of 0.20 μmol/L, which is 1460 times below the value found for gallic acid (**Table 2**). Quantitative analysis of the astringent compounds in group I revealed the highest concentrations for gallic acid, furan-2-carboxylic acid, and (*E*)-caftaric acid with 795, 220, and 130 μmol/L, respectively. The other phenolic acids were present in somewhat lower amounts ranging from 11.1 to 80.4 μmol/L, whereas the concentrations of the flavon-3-ol glucosides and dihydroflavon-3-ol rhamnosides were found to be between 0.31 μmol/L for quercetin-3-*O*-β-D-galactopyranoside and 8.42 μmol/L for isorhamnetin-3-*O*-β-D-glucopyranoside (**Table 2**). As the exact molecular mass of the HMW fraction cannot be determined, its concentration was expressed in grams per liter. Compared to all of the low molecular weight taste compounds in group I such as gallic acid (0.00135 g/L), these HMW components were present in the red wine in by far the highest concentration of 5.45 g/L (**Table 2**).

To benchmark the compounds in group I in their possible sensory impact in the red wine, the DoT factors were calculated as the ratio of the concentration to the taste recognition threshold of each individual compound (*27*). The results revealed only 7 of the 18 compounds to have DoT factors above 1.0 and were, therefore, suggested to contribute to the taste of the red wine (**Table 2**). By far the highest DoT factor of 247.7 was determined for the HMW fraction, followed by syringetin-3-*O*-β-D-glucopyranoside and (*E*)-caftaric acid judged with DoT factors of 27.0 and 8.1, respectively, and isorhamnetin-3-*O*-β-D-glucopyranoside (3.4), gallic acid (2.7), dihydroquercitin-3-*O*-α-L-rhamnopyranoside (1.5), and furan-2-carboxylic acid (1.4) with somewhat lower DoT factors (**Table 2**). For all of the other compounds in group I their concentrations in red wine did not exceed their sensory threshold, thus suggesting that these molecules might not play a major role in the taste of the red wine.

Tastant group II, summarizing all bitter tasting molecules, contained six monomeric to oligmeric flavan-3-ols and seven phenolic acid ethyl esters, as well as eight bitter tasting L-amino acids (**Table 2**). The flavan-3-ols primarily induced a puckering astringent sensation in the oral cavity, followed by a distinct bitterness perceived at somewhat higher concentrations. The taste threshold concentrations for the astringency of the flavan-3-ols were in the same range as found for the astringent phenolic acids of group I (**Table 2**). The astringent taste threshold

Table 2. Taste Qualities, Taste Thresholds, Concentrations, and Dose-over-Threshold (DoT) Factors of Sensory Active Nonvolatiles in Amarone Red Wine

taste compound	TC[a] (μmol/L)	concn (μmol/L)	DoT factor[b]
	Group I: Nonbitter Astringent Compounds		
flavonol-3-ol glycosides (velvety, silky astringency)			
syringetin-3-*O*-β-D-glucopyranoside	0.20	5.38	27.0
isorhamnetin-3-*O*-β-D-glucopyranoside	2.48	8.42	3.4
dihydroquercetin-3-*O*-α-L-rhamnopyranoside	3.70	5.71	1.5
quercetin-3-*O*-β-D-galactopyranoside	0.43	0.31	0.7
dihydrokaempferol-3-*O*-α-L-rhamnopyranoside	4.81	1.50	0.3
quercetin-3-*O*-β-D-glucuropyranoside	1.97	0.38	0.2
phenolic acids and furan acids (puckering astringent)			
(*E*)-caftaric acid	16	130	8.1
gallic acid	292	795	2.7
furan-2-carboxylic acid	160	220	1.4
caffeic acid	72	54.8	0.8
p-coumaric acid	139	80.4	0.6
ferulic acid	67	11.1	0.2
protocatechuic acid	206	31.2	0.2
syringic acic	263	48.1	0.2
vanillic acid	315	31.0	0.1
p-hydroxybenzoic acid	665	14.6	<0.1
gallic acid methyl ester	232	12.8	<0.1
polymers (puckering astringent)			
HMW fraction (>5 kDa)	22.0 [mg/L]	5.45 [g/L]	247.7
	Group II: Bitter Compounds		
flavan-3-ols (bitter and puckering astringent)			
(+)-catechin	410[c]/1000[d]	57.6	0.2[c]/<0.1[d]
(−)-epicatechin	930[c]/930[d]	27.6	0.1[c]/<0.1[d]
procyanidin B1	240[c]/400[d]	10.7	<0.1[c]/<0.1[d]
procyanidin B2	190[c]/485[d]	4.2	<0.1[c]/<0.1[d]
procyanidin B3	200[c]/500[d]	2.9	<0.1[c]/<0.1[d]
procyanidin C1	300[c]/400[d]	1.6	<0.1[c]/<0.1[d]
phenolic acid ethyl esters (bitter and puckering astringent)			
gallic acid ethyl ester	185[c]/2200[d]	153	0.8[c]/<0.1[d]
p-coumaric acid ethyl ester	143[c]/715[d]	24.6	0.4[c]/<0.1[d]
syringic acid ethyl ester	18[c]/576[d]	3.8	0.2[c]/<0.1[d]
vanillic acid ethyl ester	125[c]/1500[d]	10.8	0.1[c]/<0.1[d]
caffeic acid ethyl ester	277[c]/1100[d]	16.5	<0.1[c]/<0.1[d]
ferulic acid ethyl ester	67[c]/710[d]	1.1	<0.1[c]/<0.1[d]
protocatechuic acid ethyl ester	49[c]/1000[d]	4.1	<0.1[c]/<0.1[d]
amino acids (bitter)			
L-histidine	45000	107	<0.1
L-valine	20000	280	<0.1
L-isoleucine	10000	174	<0.1
L-leucine	11000	195	<0.1
L-lysine	80000	191	<0.1
L-phenylalanine	45000	171	<0.1
L-tyrosine	4000	113	<0.1
L-arginine	75000	2834	<0.1
	Group III: Sweet Compounds		
aldoses and ketoses			
fructose	10200	25642	2.5
glucose	18000	6719	0.4
arabinose	17700	2200	0.1
galactose	50000	1136	<0.1
xylose	12500	604	<0.1
rhamnose	11800	308	<0.1
alditols			
glycerol	81200	215092	2.6
1,2-propanediol	44200	13929	0.3
inositol	17700	3606	0.2
mannitol	40000	3566	0.1
arabitol	43100	1182	<0.1
erythritol	36300	2786	<0.1
sorbitol	33800	494	<0.1
ribitol	45300	66	<0.1
amino acids			
L-proline	25000	16095	0.6
L-alanine	12000	940	0.1
glycine	25000	1707	0.1
L-methionine	5000	43	<0.1
L-serine	25000	119	<0.1
L-threonine	35000	106	<0.1

Table 2. Continued

taste compound	TC[a] (μmol/L)	concn (μmol/L)	DoT factor[b]
	Group IV: Compounds Imparting Sourness/Saltiness		
organic acids			
tartaric acid	292	7290	24.8
galacturonic acid[f]	643	9122	14.2
acetic acid	1990	12278	6.0
succinic acid	900	2695	3.0
malic acid	3690	6849	1.9
lactic acid	15480	22928	1.5
citric acid	2600	1787	0.7
glutaric acid	3125	1094	0.3
formic acid	4338	286	<0.1
isocitric acid	5300	208	<0.1
maleic acid	3000	80	<0.1
(*Z*)-aconitic acid [c]	500	16	<0.1 (32[c])
(*E*)-aconitic acid[c]	500	9.2	<0.1 (19[c])
cations/anions			
potassium[g]	12600	33150	2.6
magnesium[g]	3200	8247	2.0
ammonium[g]	5000	6432	1.2
phosphate[h]	7500	6448	0.8
calcium[g]	3100	2053	0.7
sodium[g]	3900	2837	0.6
chloride[i]	3900	1250	0.3
	Group V: Umami Compounds		
L-glutamic acid	1200	578	0.5
L-asparagine	50000	196	0.1
L-aspartic acid	20000	509	<0.1
L-glutamine	50000	95	<0.1

[a] Taste threshold concentrations (TC) were determined in bottled water by means of a triangle test for bitter, sweet, sour, salty, and umami compounds and by means of the half-tongue test for astringent compounds. [b] The DoT factor is calculated as the ratio of the concentration and taste threshold. [c] Taste threshold for astringency is 0.5 μmol/L. The DoT factors for astringency are given in parentheses. [d] Taste threshold or DoT factor for bitterness. [e] Taste threshold for sourness is 500 μmol/L. [f] Taste threshold or DoT factor for astringency and sourness. [g] Taste threshold and DoT factor determined for the corresponding chloride salt. [h] Taste threshold and DoT factor determined for the corresponding potassium salt. [i] Taste threshold and DoT factor determined for the corresponding sodium salt.

decreased from the monomeric (−)-epicatechin to the dimeric procyanidins B1, B2, and B3 and then increased slightly to the procyanidin trimer C1, whereas the bitter taste thresholds of the procyanidins were found to be in a rather narrow range between 400 and 500 μmol/L. These data are in contradiction to a previous paper showing lower threshold concentrations of 160 and 30 μmol/L for (+)-catechin and procyanidin B3 (*7*), but are in good agreement with the sensory thresholds recently reported for the same compounds isolated from cocoa nibs (*24*). Sensory analysis of the phenolic acid ethyl esters revealed a puckering astringency with similar threshold concentrations as found for the phenolic acids present in group I. Depending on their chemical structure, the bitter taste thresholds of the phenolic acid ethyl esters were 5−20 times above the threshold concentrations found for astringency (**Table 2**). Whereas the phenolic compounds in group II exhibited besides astringency also bitterness, the amino acids were judged to impart exclusively a bitter taste sensation, with taste thresholds above 4000 μmol/L as found for L-tyrosine.

Quantitative analysis of the tastants in group II revealed the amino acids to be present in high concentrations; for example, the amounts of L-arginine were 2834 μmol/L (**Table 2**). All of the other bitter amino acids were present in amounts between 107 and 195 μmol/L, which is the same range as found for the bitter gallic acid ethyl ester (153 μmol/L). The other phenolic acid ethyl ester and the flavan-3-ols were present in lower amounts ranging from 1.1 μmol/L found for ferulic acid ethyl ester to 57.6 μmol/L found for (+)-catechin (**Table 2**).

Calculation of DoT factors clearly demonstrated that none of the compounds in group II reached a value of 1.0, thus indicating that the single compounds might not play an important role in the taste of the red wine. The ethyl esters of gallic acid, *p*-coumaric acid, and syringic acid as well as (+)-catechin reached DoT factors of 0.8, 0.4, and 0.2 for astringency, whereas all of the other compounds were >10 times below their threshold concentrations (**Table 2**). Although taste dilution analysis identified the phenolic acid ethyl esters as potential bitter compounds in Amarone red wine (*23*) and multiple literature data pinpointed procyanidins as bitter contributors (*4, 11, 12, 19, 20*), these compounds are present in the red wine in subthreshold concentrations only.

Tastant group III, summarizing the sweet compounds, consisted of six aldoses and ketoses, eight alditols, and six amino acids (**Table 2**). The taste thresholds of the aldoses and ketoses were found to range between 10.2 and 50 mmol/L with the lowest threshold value found for D-fructose. The sweet amino acids were evaluated with similar recognition thresholds as the sugars with the lowest value of 5.0 mmol/L found for L-methionine. When compared to the amino acids, the aldoses and ketoses as well as the alditols exhibited up to 4 times higher recognition thresholds. Among the group of alditols, the highest sweet taste threshold of 81 mmol/L was found for glycerol, whereas *myo*-inositol imparted sweetness already at the lowest threshold concentration of 17.7 mmol/L (**Table 2**).

Quantitative studies revealed glycerol and D-fructose with concentrations of 215 and 25.6 mmol/L as the predominating compounds in tastant group III. Calculation of DoT factors demonstrated that both compounds exceeded their sweet threshold concentration in red wine; for example, DoT factors of 2.6 and 2.5 were determined for glycerol and D-fructose, respectively. In addition, the concentrations of L-proline (0.6) and D-glucose (0.4) were close to their sweetness thresholds, thus indicating that these compounds might contribute to a lower extent to the sweetness of the wine.

Tastant group IV, summarizing all of the compounds eliciting a sour or salty taste, consisted of 13 organic acids, as well as 7 inorganic cations and anions (**Table 2**). Determination of the taste thresholds of these compounds showed a surprisingly low taste threshold of 0.5 μmol/L found for the puckering astringent sensation induced by (*E*)- and (*Z*)-aconitic acid, whereas a clear sour taste impression was recorded above 500 μmol/L (**Table 2**). L-Tartaric acid was found with the lowest threshold for sourness (292 μmol/L), whereas L-lactic acid imparted sourness above the rather high threshold concentration of 15 mmol/L. Quantification of organic acids revealed by far the highest concentration for L-lactic acid (22.9 mmol/L), followed acetic acid (12.3 mmol/L) and D-galacturonic acid (9.1 mmol/L) (**Table 2**). The content of the other organic acids such as L-tartaric acid, succinic acid, and L-malic acid ranged between 1 and 8 mmol/L. Calculation of DoT factors revealed D-galacturonic acid and L-tartaric acid as important sour stimuli in the wine as their concentrations exceeded their taste threshold by a factor of >10 (**Table 2**). In addition, acetic acid, succinic acid, L-malic acid, and L-lactic acid were evaluated with DoT factors above 1.0. Comparatively small concentrations were found for (*Z*)- and (*E*)-aconitic acid, respectively, thus excluding any contribution to the sour taste of the wine. However, due to their low threshold concentration for astringency (0.5 μmol/L), high DoT factors of 32.0 and 18.6 for astringency were calculated for (*Z*)- and (*E*)-aconitic acid, thus implying that these acids might contribute to the astringency of the wine.

Among the cations and anions, potassium and phosphate were quantitatively predominating with concentrations of 33150 and 6448 μmol/L, respectively (**Table 2**). Determination of the DoT factors revealed relatively high values of 2.6 and 2.0 for potassium and magnesium evaluated as their corresponding chlorides, whereas sodium and calcium chloride did not exceed their sensory thresholds.

Finally, L-glutamic acid, L-aspartic acid, L-glutamine, and L-asparagine were summarized to give the umami group V (**Table 2**). Sensory evaluation and quantification revealed that none of these amino acids reached its taste threshold, thus implying that a contribution of these compounds to red wine taste is rather unlikely.

Re-engineering of the Nonvolatile Sensometabolome of Red Wine. To confirm the results of the instrumental analysis and to demonstrate that the compounds identified can create the typical taste of the red wine, taste re-engineering experiments were performed.

First, an aqueous taste recombinant was prepared by solubilizing all 82 compounds summarized in groups I−V (**Table 2**), each in the concentration determined in the red wine, in 15% aqueous ethanol (pH 3.8). The color of the samples was not adjusted, because the presence of the deeply colored HMW fraction (<5 kDa) in the recombinant did not allow the sensory differentiation between recombinant and wine by color. The sensory panel was then asked to evaluate the taste profile of both samples by scoring the taste descriptors given in **Table 1** on a scale from 0 (not detectable) to 5 (strong taste impression). Sensory evaluation of the total taste recombinant as well as the authentic wine revealed the highest impact for the puckering astringent sensation evaluated with an intensity of 4.1 or 4.0, respectively, as well as for body/mouthfulness judged with intensities of 3.8 and 4.0 (**Table 1**). Also, bitterness, sweetness, and sourness of the recombinant, judged with intensities of 1.3, 1.9, and 3.2, were rather close to those of the red wine evaluated with scores of 1.5, 2.0, and 3.0, respectively, and the velvety astringent onset matched completely. As the taste profile of the recombinant was very close to that of the authentic wine, the trained panelists concluded that the typical taste of the wine could be completely reconstituted by the blend of the 82 components present in groups I−V (**Table 2**).

Taste Omission Experiments. To investigate the taste contribution and relevance of the individual taste compounds as well as putative interactions between different tastant groups, systematic taste omission experiments were performed. To achieve this, individual taste recombinants lacking either in one tastant group or in one or more individual taste compounds were evaluated by means of triangle tests using two samples of the complete taste recombinant as the control. Those panelists who detected the taste difference correctly were asked to rate the intensity of the taste descriptors puckering astringency, velvety astringency, bitterness, sweetness, sourness, and mouthfulness/body on a five-point scale (**Table 3**).

First, single tastant groups were omitted from the total taste recombinant, and the sensory impact of this tastant omission was evaluated by a taste profile analysis (**Table 3**). The omission of group I, containing the astringent flavonol and dihydroflavonol glycosides and the phenolic acids, as well as the HMW fraction (<5 kDa), was detected by all sensory panelists and was found to induce a dramatic loss of the velvety astringent onset (2.5 → 0.5) as well as the puckering astringent offset (4.1 → 0.5) (**Table 3**; expt 1). Additional partial recombinants were prepared lacking in either the velvety astringent flavonol and dihydroflavonol glycosides (expt 2), the phenolic acids (expt 3), or the polymers (expt 4) and were compared in a triangle test to two samples of the total recombinant. Seven of eight panelists detected the omission of the flavonol and dihydroflavonol glycosides and observed a strong decrease of the velvety astringent onset (2.5 → 0.6) as well as a slight decrease in the puckering astringent offset (**Table 3**). Whereas the omission of the group of phenolic acids was not significantly detected by the sensory panel, the lack of the polymeric fraction was significantly identified by all sensory panelists and was found to induce a strong decrease of the puckering astringency (4.1 → 0.4) besides a slight reduction (0.3 unit) for the velvety astringent sensation (**Table 3**). These data demonstrate that the flavon-3-ol and dihydroflavon-3-ol glycosides are the main contributors to the velvety astringent taste impression, whereas the puckering astringent offset is mainly due to the polymers (>5 kDa) evaluated with the highest DoT factor (**Table 2**), thus confirming earlier suggestions in the literature (*19, 28, 29*). Surprisingly, the phenolic acids seem not to contribute to the taste of the red wine, although DoT factors of <1.0 were determined for (*E*)-caftaric acid and gallic acid, respectively (**Table 2**).

In a second set of experiments, group II containing the bitter/astringent flavan-3-ols, phenolic acid ethyl esters, and the bitter amino acids was omitted from the taste recombinant (**Table 3**; expt 5). This partial recombinant could be differentiated from the total recombinant by all eight panelists, who found nearly a complete loss of bitterness (1.5 → 0.1) without influencing the astringent taste sensation at all. It is interesting to note that the omission of these compounds diminished the bitterness, although the DoT factors of the individual compounds were <0.1 (**Table 2**). To answer the question as to which group of compounds is responsible for evoking the bitter taste, additional partial recombinants were prepared lacking either the group of flavan-3-ols (expt 6), phenolic acid ethyl esters (expt 7), or the bitter tasting amino acids (expt 8; **Table 3**). Whereas the omission of the bitter amino acids could not be detected, the lack of the flavan-3-ols or the phenolic acid ethyl esters,

Table 3. Influence of the Omission of Tastant Groups or Individual Taste Compounds on the Taste Profile of the Taste Recombinant

expt no.	omission of	no.[a]	description of taste difference[b] (change in intensity)
1	**group I (astringent)**[c]	8	loss of puckering astringent offset (4.1 → 0.5) loss of velvety astringent onset (2.5 → 0.5) increase in sweetness (1.9 → 2.0)
2	flavonol-/dihydroflavonol-glycosides	7	loss of puckering astringent offset (4.1 → 3.8) loss of velvety astringent onset (2.5 → 0.6)
3	phenolic acids	1	no difference detectable
4	polymers (>5 kDa)	8	loss of puckering astringent offset (4.1 → 0.5) loss of velvety astringent onset (2.5 → 2.2)
5	**group II (bitter/astringent)**[c]	8	loss of bitterness (1.5 → 0.1)
6	flavan-3-ols	7	decrease in bitterness (1.5 → 1.0)
7	phenolic acid ethyl esters	7	decrease in bitterness (1.5 → 0.6)
8	amino acids	0	no difference detectable
9	**group III (sweet)**[c]	8	loss of sweetness (1.9 → 0.1) loss of mouthfulness/body (3.8 → 0.1) increase in puckering astringent offset (4.1 → 4.5) increase in velvety astringent onset (2.5 → 2.8) increase in bitterness (1.3 → 1.5) increase in sourness (3.2 → 3.5)
10	aldoses, ketoses	8	decrease in sweetness (1.9 → 1.0) decrease in mouthfulness/body (3.8 → 3.5) increase in puckering astringency (4.1 → 4.4) increase in sourness (3.2 → 3.5)
11	aldoses, ketoses (DoT ≤ 0.1)	0	no difference detectable
12	amino acids	6	decrease in sweetness (1.9 → 1.4) increase in sourness (3.2 → 3.4)
13	amino acids (DoT ≤ 0.1)	0	no difference detectable
14	alditols	8	decrease in sweetness (1.9 → 0.8) loss of mouthfulness/body (3.8 → 0.5) increase in puckering astringent offset (4.1 → 4.5) increase in velvety astringent onset (2.5 → 2.6) increase in bitterness (1.3 → 1.4) increase in sourness (3.2 → 3.5)
15	alditols (DoT ≤ 0.5)	6	decrease in sweetness (1.9 → 1.1) decrease in mouthfulness/body (3.8 → 1.9) increase in puckering astringency (4.1 → 4.5)
16	alditols (DoT < 0.1)	0	no difference detectable
17	**group IV (sour/salty)**	8	decrease in sourness (3.2 → 1.0) decrease in puckering astringent offset (4.1 → 3.0) increase in velvety astringent onset (2.5 → 2.6)
18	organic acids	8	decrease in sourness (3.2 → 1.0) decrease in puckering astringent offset (4.1 → 3.0) increase in velvety astringent onset (2.5 → 2.6)
19	organic acids (DoT < 1.0)	0	no difference detectable
20	cations/anions	7	increase in sourness (3.2 → 4.0)
21	cations/anions (DoT < 1.0)	0	no difference detectable
22	**group V**	0	no difference detectable

[a] Number of individuals out of eight panelists detecting the recombinant lacking certain tastants by means of a triangle test. [b] Partial recombinants lacking certain tastants were presented to the panel by means of a triangle test. If sample was correctly chosen, differences in taste intensities were evaluated on a scale of 0 (not detectable) to 5 (strong taste impression). [c] Components of each group are listed in.

respectively, was correctly identified by seven of the eight panelists and was evaluated to induce a decrease in bitterness by 0.5 and 0.9 unit, respectively (**Table 3**). On the basis of these data, it can be concluded that subthreshold concentrations of phenolic acid ethyl esters and flavanols contribute to red wine bitterness.

In the third set of experiments, the sweet-tasting group III, containing sugars, amino acids, and alditols, were omitted from the total recombinant. As given in **Table 3** (expt 9), the lack of this fraction was correctly detected by all eight panelists and was found to induce an almost complete loss of sweetness (1.9 → 0.1) as well as of the mouthfulness/body (3.8 → 0.1). In addition, this partial recombinant was perceived with higher scores for the puckering astringent offset (+0.4), the velvety astringent onset (+0.3), sourness (+0.3), and bitterness (+0.2). The panelists reported that the typical taste profile of the red wine was completely out of balance. To elucidate the taste contribution of the individual compound groups, additional partial recombinants were prepared lacking in either aldoses/ketoses (expts 10 and 11), sweet amino acids (expts 12 and 13), or alditols (expts 13−16), respectively (**Table 3**). The recombinant lacking the aldoses/ketoses (expt 10) was clearly distinguished from the total recombinant by all members of the panel, who judged the partial recombinant to be lower in sweetness (−0.9) and mouthfulness/body (−0.3) and higher in puckering astringency (+0.4) and sourness (+0.3), respectively. As the omission of all of the sweet amino acids was detected by six of eight panelists and was found to induce a decrease in sweetness (−0.5) and an increase in sourness (+0.2) (expt 12), but the lack of the amino acids judged with DoT factors ≤0.1 (expt 13) was not detectable by a single panelist, the L-proline evaluated with a DoT factor of >0.5 was identified as an important sweet taste contributor. Interestingly, omission of the alditols strongly influenced the taste profile (**Table 3**, expt 14) and, in particular, the perceived mouthfulness/body (−3.3 units) as well as the intensity of sweetness (−1.1 units) were strongly decreased. In contrast, the astringent, bitter, and sour taste impressions were judged to be slightly intensified. These data

clearly demonstrate the alditols as the key ingredients for the mouthfulness/body of the red wine.

To further narrow the number of alditols, additional partial recombinants were prepared lacking the alditols evaluated with DoT factors of either ≤0.5 or <0.1, respectively. Six of eight panelists were able to detect the omission of the alditols judged with DoT factors ≤0.5 (expt 15) and to describe the difference to the total recombinant as less sweet (−0.8) and decreased mouthfulness/body (−1.9). In contrast, the lack of the alditols judged with DoT factors <0.1 (expt 16) was not detectable, thus excluding them as sensory-active key components. In addition, it was previously reported that soluble sugars and glycerol contribute to wine taste (*30*). 1,2-Propandiol, *myo*-inositol, and D-mannitol were found to contribute to mouthfulness/body and, among the sweet amino acids, L-proline with a DoT factor of 0.6 (**Table 2**) was demonstrated to contribute to the sweetness of the wine.

In a fourth set of experiments, the sour/salty group IV, containing organic acids, anions, and cations, was omitted from the total recombinant. All eight panelists determined this partial recombinant to be significantly less sour (−2.2) when compared to the total recombinant (**Table 3**, expt 17). In addition, a decrease of the puckering astringent taste sensation by 1.1 units was observed. Exactly the same sensory results were found after removal of just the group of organic acids from the recombinant (expt 18), thus being well in line with the high DoT factors calculated for these compounds. The influence of the organic acids on astringency confirms earlier literature studies reporting on the ability of organic acids to impart and/or modify the perception of astringency (*16, 17*). Omission of just the organic acids evaluated with DoT factors of <1.0 (expt 19) was not detectable at all, thus demonstrating L-tartaric acid, D-glucuronic acid, acetic acid, succinic acid, L-malic acid, and L-lactic acid as the key stimuli of the sour taste of the red wine. The partial recombinant lacking the cation and anions was significantly detected by seven of the eight panelists and was described by an increase of the sour taste impression by 0.8 unit, thus demonstrating that these salts have a suppressive effect in the perception of sourness (expt 20). Omission of the salt/anions evaluated with DoT factors of <1.0 (expt 21) did not influence the taste of the recombinant, thus demonstrating the chlorides and phosphates of potassium, magnesium, and ammonium as taste contributors.

Finally, a partial recombinant lacking the umami-like-tasting group V was prepared (expt 22), but none of the panelists could detect any taste difference from the total recombinant, thus demonstrating that the umami compounds did not contribute to the taste of the red wine (**Table 3**).

As the omission experiments allowed a separation of the molecules contributing to the overall taste of wine from those compounds that were not found to be sensorially active in their "natural" concentrations, a reduced taste recombinant A was prepared containing only the key taste ingredients, namely, the six velvety astringent flavonol and dihydroflavonol glycosides, the puckering astringent polymeric fraction (>5 kDa), the six bitter tasting flavan-3-ols, the seven bitter phenolic acid ethyl esters, the sweet tasting compounds D-glucose, D-fructose, L-proline, glycerol, 1,2-propanediol, and *myo*-inositol, and the chlorides and phosphates of potassium, magnesium, and ammonium phosphate, as well as the six organic acids evaluated with DoT factors of ≥1.0 (**Table 2**). By means of a triangle test, the taste profile of the reduced recombinant A (35 compounds) could not be differentiated from that of the total recombinant (82 compounds) (**Table 4**), thus demonstrating these 35 compounds as the key taste compounds responsible for the typical taste of Amarone red wine.

Table 4. Taste Profile Analysis of the Total Taste Recombinant and the Partial Recombinants A and B

taste quality	intensities of individual taste qualities in[a]		
	total recombinant[b] (82 cmpds)	partial recombinant A[c] (35 cmpds)	partial recombinant B[d] (15 cmpds)
puckering astringent offset	4.1	4.1	4.0
velvety astringent onset	2.5	2.5	2.0
mouthfulness, body	3.8	3.8	2.8
bitter	1.3	1.3	0
sweet	1.9	1.9	1.5
sour	3.2	3.2	3.2
salty	0	0	0
umami	0	0	0

[a] Intensities were rated on a scale from 0 (not detectable) to 5 (strong taste impression). [b] The total taste recombinant consisted of the 82 compounds identified (**Table 2**), solubilized in 15% aqueous ethanol (pH 3.8). [c] The partial taste recombinant A consisted of the 35 compounds judged as important taste compounds based on the omission experiments (**Table 3**). [d] The partial taste recombinant B consisted of the 15 compounds evaluated with DoT factors < 1.0.

To further narrow the number of key tastants, another reduced taste recombinant B was prepared from 15 compounds by omitting from recombinant A all of the compounds evaluated with DoT factors of <1.0. Comparison of the taste profile of the recombinant B containing syringetin-3-*O*-*β*-D-glucopyranoside, isorhamnetin-3-*O*-*β*-D-glucopyranoside, dihydroquercetin-3-*O*-*β*-D-glucopyranoside, the polymeric fraction (>5 kDa), D-fructose, glycerol, L-tartaric acid, D-galacturonic acid, acetic acid, succinic acid, L-malic acid, L-lactic acid, potassium chloride, magnesium chloride, and ammonium phosphate to the taste profile of the total recombinant revealed the lack of any bitterness in this reduced recombinant (**Table 4**). This finding clearly indicates that subthreshold concentrations of the polyphenol acid ethyl esters and the flavan-3-ols are responsible for the bitterness of the red wine. In addition, the sweetness (−0.4) and the mouthfulness/body (−1.0) were slightly reduced, thus demonstrating that besides the glycerol also 1,2-propanediol and *myo*-inositol contribute to the mouthfulness of the red wine. In conclusion, quantitative studies, followed by taste re-engineering and carefully planned omission experiments, allowed for the first time a comprehensive identification and evaluation of the key sensometabolites of a red wine.

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