DOI:10.1021/jf9044606

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Quantitative Studies on the Influence of the Bean Roasting Parameters and Hot Water Percolation on the Concentrations of Bitter Compounds in Coffee Brew

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To investigate the influence of roasting time and temperature on the degradation of the bitter precursors 3-*O*-caffeoyl quinic acid (**1**), 5-*O*-caffeoyl quinic acid (**2**), and 4-*O*-caffeoyl quinic acid (**3**) as well as the formation of bitter tastants during coffee roasting, we prepared coffee brews from beans roasted either at 260 °C for 60−600 s or for 240 s at 190−280 °C. By means of HPLC-UV/vis and HPLC-MS/MS, bitter-tasting monocaffeoyl quinides (**4**−**8**), dicaffeoyl quinides (**9**−**11**), and 4-vinylcatechol oligomers (**12**−**20**) as well as the parent bitter precursors **1**−**3** were quantitatively analyzed in these brews. Quinides **4**−**11**, exhibiting a coffee-typical bitter taste profile, were found to be preferentially formed under slight to medium roasting degrees and were observed to be degraded again to generate harsh bitter-tasting 4-vinylcatechol oligomers under more severe roasting conditions, thus matching the change in bitter taste quality observed by means of sensory studies. In addition, quantitative studies of the release profile of bitter compounds from ground coffee upon water percolation revealed that compounds **1**−**8** were rapidly extracted, dicaffeoyl quinides **9**−**11** were released rather slowly, and, in particular, compounds **12**−**17** were found to show strong retention to the ground coffee material. These data imply that the knowledge-based control of the roasting and/or the extraction conditions might be helpful in tailoring the bitter taste signature of coffee beverages.

KEYWORDS: Coffee; bitter compounds; bitter taste; caffeoyl quinic acids; caffeoyl quinides; phenyl indanes; coffee roasting

INTRODUCTION

Freshly brewed coffee is appreciated by consumers all over the world because of to its stimulating effect, its attractive aroma, and its characteristic taste centering around bitterness and sourness. Screening of the coffee volatiles for odor activity by means of gas chromatography and olfactometry, quantitation of the most odor-active molecules by using stable isotope dilution analyses, and aroma reconstitution experiments impressively demonstrated that only ~25 of more than 1000 volatiles contribute to the overall aroma of a freshly prepared coffee beverage (*1*−*4*). In contrast, the knowledge of the molecules inducing the typical bitter taste of coffee beverages is still far from being comprehensive.

A series of studies performed over the past 30 years ago focused on the identification of the bitter-tasting molecules in coffee and suggested the alkaloids caffeine and trigonelline (*5*) as well as thermally generated compounds such as, for example, furfuryl alcohol (*6*), 5-hydroxymethyl-2-furanaldehyde (*7*), pyrazines (*7*), and diketopiperazines (*8*) as putative candidates for the key bitter compounds in coffee. However, systematic sensory-guided fractionation of a coffee beverage as well as suitable model systems followed by LC-MS/MS and one- and two-dimensional NMR studies revealed that during coffee roasting 3-*O*-caffeoyl quinic acid (**1**), 5-*O*-caffeoyl quinic acid (**2**), and 4-*O*-caffeoyl quinic acid (**3**), respectively, the major polyphenols in raw coffee, are thermally transformed into the bitter-tasting caffeoyl quinic acid lactones 5-*O*-caffeoyl-*muco*-γ-quinide (**4**), 3-*O*-caffeoyl-γ-quinide (**5**), 4-*O*-caffeoyl-*muco*-γ-quinide (**6**), 5-*O*-caffeoyl-*epi*-δ-quinide (**7**), 4-*O*-caffeoyl-γ-quinide (**8**), 3,4-*O*-dicaffeoyl-γ-quinide (**9**), 4,5-*O*-dicaffeoyl-*muco*-γ-quinide (**10**), and 3,5-*O*-dicaffeoyl-*epi*-δ-quinide [**11** (**Figure 1**)] (*9*). Furthermore, a series of lingering and harsh bitter-tasting 4-vinylcatechol oligomers [**12**−**20** (**Figure 1**)] were recently identified in coffee beverages and were demonstrated to be formed upon thermal degradation of 5-*O*-caffeoyl quinic acid [**2** (**Figure 1**)] and caffeic acid, respectively (*10*, *11*). Although some preliminary data have been reported on the degradation of *O*-caffeoyl quinic acids during roasting of coffee beans (*12*−*16*), systematic studies of the influence of the roasting parameters on the bitter-tasting caffeoyl quinides **4**−**11** and harsh bitter-tasting 4-vinylcatechol oligomers **12**−**20** are lacking.

The objective of this investigation was, therefore, to quantitatively determine the amounts of bitter-tasting *O*-caffeoyl quinides **4**−**11** and 4-vinylcatechol oligomers **12**−**20** as well as the parent *O*-monocaffeoyl quinic acids **1**−**3** in beverages freshly prepared from coffee beans varying in terms of roasting degree. In addition,

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 Published on Web 02/24/2010

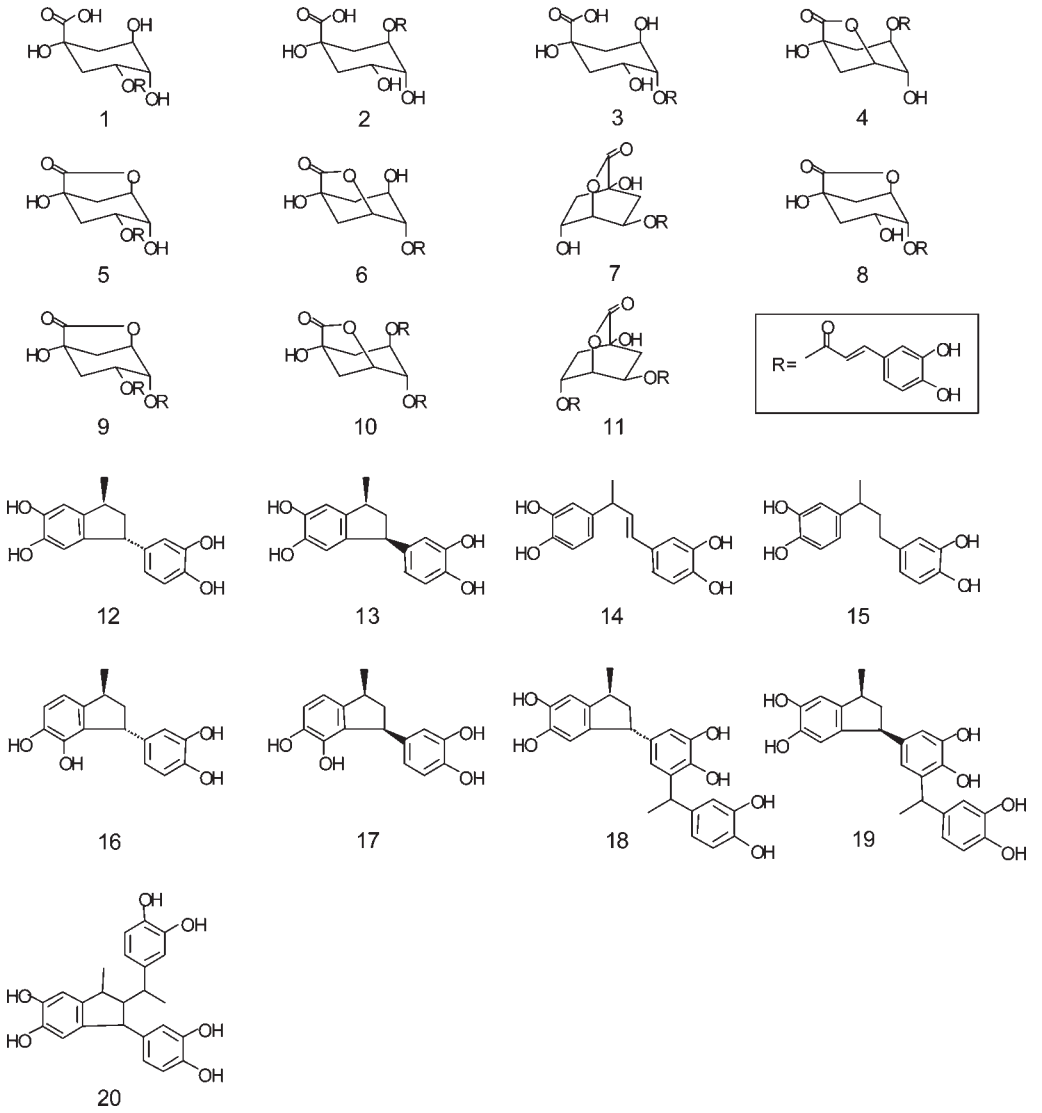

Figure 1. Chemical structures of 3-*O*-caffeoyl quinic acid (**1**), 5-*O*-caffeoyl quinic acid (**2**), 4-*O*-caffeoyl quinic acid (**3**), 5-*O*-caffeoyl-*muco*-γ-quinide (**4**), 3-*O*-caffeoyl-γ-quinide (**5**), 4-*O*-caffeoyl-*muco*-γ-quinide (**6**), 5-*O*-caffeoyl-*epi*-δ-quinide (**7**), 4-*O*-caffeoyl-γ-quinide (**8**), 3,4-*O*-dicaffeoyl-γ-quinide (**9**), 4,5-*O*-dicaffeoyl-*muco*-γ-quinide (**10**), 3,5-*O*-dicaffeoyl-*epi*-δ-quinide (**11**), *trans*-5,6-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane (**12**), *cis*-5,6-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane (**13**), *trans*-1,3-bis(3′,4′-dihydroxyphenyl)-1-butene (**14**), 1,3-bis(3′,4′-dihydroxyphenyl)butane (**15**), *trans*-4,5-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane (**16**), *cis*-4,5-dihydroxy-1-methyl-3-(3′,4′-dihydroxyphenyl)indane (**17**), *trans*-5,6-dihydroxy-1-methyl-3-{3′,4′-dihydroxy-5′-[1-(3′′,4′′-dihydroxyphenyl)-1-ethyl]phenyl}indane (**18**), *cis*-5,6-dihydroxy-1-methyl-3-{3′,4′-dihydroxy-5′-[1-(3′′,4′′-dihydroxyphenyl)-1-ethyl]phenyl}indane (**19**), and 5,6-dihydroxy-1-methyl-2-[1-(3′,4′-dihydroxyphenyl)-1-ethyl]-3-(3′′,4′′-dihydroxyphenyl)indane (**20**).

the release of these compounds upon hot water percolation should be investigated on a quantitative basis.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: D-(−)-quinic acid, *p*-toluenesulfonic acid monohydrate, chloro formic acid 2,2,2-trichloroethyl ester, anhydrous pyridine, caffeic acid, methyl chloroformate, ammonium fomate, 5-*O*-caffeoyl quinic acid, resveratrol (Sigma Aldrich, Steinheim, Germany), sodium hydrogen carbonate, hydrochloric acid, sodium sulfate, trichloroacetic acid, sodium hydroxide, thionyl chloride, lithium chloride, sodium carbonate, sodium chloride (Merck, Darmstadt, Germany), formic acid (Grüssing, Filsum, Germany), and d_4-methanol (Euriso-Top, Saarbrücken, Germany). Solvents were of HPLC grade (Merck).

Following the protocol reported recently (*9*), 3-*O*-caffeoyl quinic acid (**1**), 5-*O*-caffeoyl quinic acid (**2**), 4-*O*-caffeoyl quinic acid (**3**), 5-*O*-caffeoyl-*muco*-γ-quinide (**4**), 3-*O*-caffeoyl-γ-quinide (**5**), 4-*O*-caffeoyl-*muco*-γ-quinide (**6**), 5-*O*-caffeoyl-*epi*-δ-quinide (**7**), and 4-*O*-caffeoyl-γ-quinide (**8**) were isolated and purified from a decaffeinated coffee or thermally treated 5-*O*-caffeoyl quinic acid. 4,5-*O*-Dicaffeoyl-*muco*-γ-quinide (**10**) and 3,5-*O*-dicaffeoyl-*epi*-δ-quinide (**11**) were isolated from thermally treated 4,5-*O*-dicaffeoyl quinic acid and 3,5-*O*-dicaffeoyl quinic acid, respectively (*9*). The coffee beans (Arabica Brazil, Santos) were provided by the coffee industry.

Preparation of a Standard Coffee Beverage. After the coffee beans had been ground with a batch mill (IKA, Staufen, Germany), aliquots (54 g) of coffee powder placed in a coffee filter (No. 4, Melitta) were percolated with boiling water until the filtrate reached a volume of 1.0 L. The coffee beverage obtained was immediately cooled to room temperature in an ice bath.

Sequential Hot Water Extraction of Ground Coffee. Aliquots (5.4 g) of ground coffee were placed into an empty solid-phase extraction tube (Phenomenex, Aschaffenburg, Germany) (volume of 60 mL), sealed with a filter paper disk, and sequentially percolated with boiling water in 10 aliquots (10 mL each) using a solid-phase extraction vacuum processor (Phenomenex). The filtrates of each of the 10 elution steps were individually collected in a volumetric flask. In addition, aliquots (5.4 g) of coffee powder were placed into a solid-phase extraction tube and percolated with

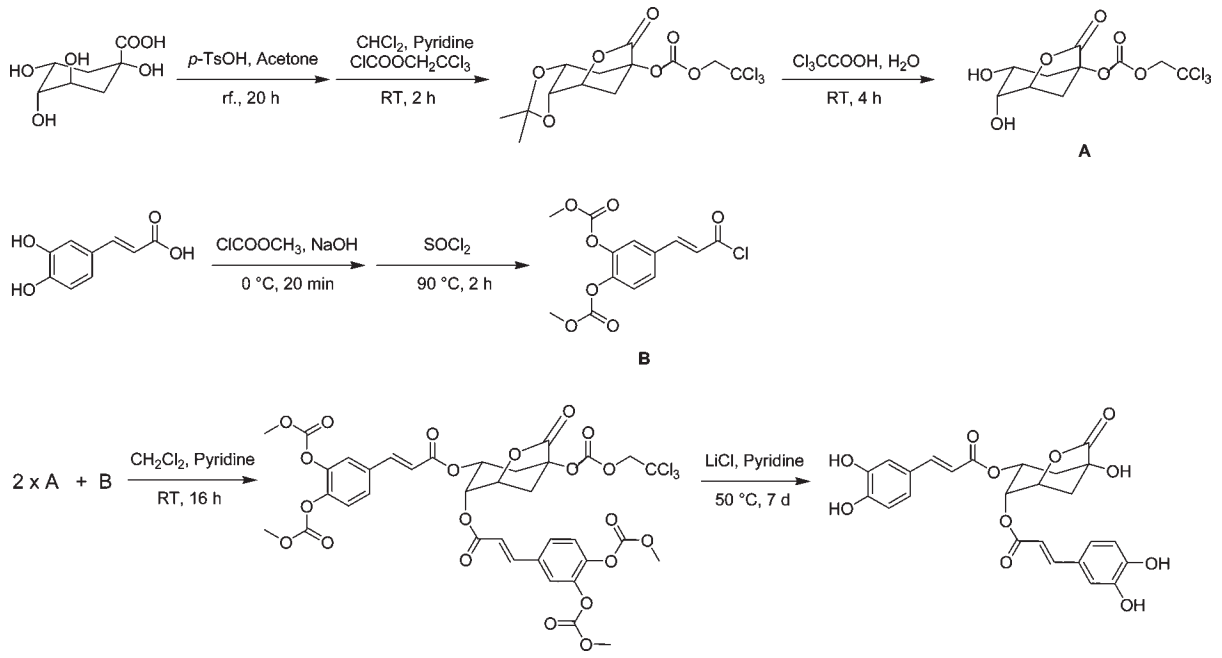

Figure 2. Reaction route used for the independent synthesis of 3,4-*O*-dicaffeoyl-γ-quinide (**9**).

hot water (100 mL), and the effluent was collected in an ice-cooled glass vial.

Synthesis of 3,4-*O*-Dicaffeoyl-γ-quinide (9). Following a literature procedure (*17*) with some modifications, D-(−)-quinic acid (156 mmol) and *p*-toluenesulfonic acid monohydrate (5.89 mmol) were suspended in dry acetone (600 mL) and refluxed for 20 h in a Soxhleth apparatus, which was equipped with an extraction thimble filled with a molecular sieve [15 g, 4 Å (Merck)]. After the sample had cooled to 5 °C, sodium hydrogen carbonate (95.2 mmol) was added and the suspension was stirred for an additional 60 min. After filtration, the solvent was removed in vacuum, the residue was taken up in dichloromethane (300 mL), and 3,4-*O*-isopropyliden-1,5-quinide was crystallized upon addition of *n*-hexane (300 mL). A solution of chloroformic acid 2,2,2-trichloroethyl ester (156 mmol) in dichloromethane (50 mL) was added dropwise to a mixture of 3,4-*O*-isopropyliden-1,5-quinide (146 mmol), pyridine (338 mmol), and dichloromethane (300 mL) at 0 °C and was, then, stirred for 2 h at room temperature. The white precipitate formed was separated by filtration and dissolved in dichloromethane (400 mL), and the solution was extracted with hydrochloric acid (1 mol/L, 2 × 150 mL), followed by water (100 mL). The organic layer was dried over Na_2SO_4 and then concentrated to 100 mL in vacuum, and upon addition of ethanol (800 mL) and cooling at 4 °C overnight, 1-*O*-(2,2,2-trichloroethoxycarbonyl)-3,4-*O*-isopropyliden-1,5-quinide precipitated and was separated by filtration. Water (6.8 mL) was added to trichloroacetic acid (374 mmol) and then heated until a clear solution was obtained to which, after the sample had cooled to room temperature, 1-*O*-(2,2,2-trichloroethoxycarbonyl)-3,4-*O*-isopropyliden-1,5-quinide (118 mmol) was added in proportions. After the sample had been stirred for 4 h, ice-cooled water (450 mL), ethyl acetate (900 mL), and then an aqueous $NaHCO_3$ solution (405 mmol in 900 mL of water) were stepwise added to the reaction mixture. The organic phase was separated and extracted with an aqueous $NaHCO_3$ solution (2% in water, 120 mL) and water (120 mL). The organic layer was dried over Na_2SO_4, the solvent removed in vacuum, and the residue dissolved in toluene (250 mL) at 70 °C. The solution was kept at 4 °C overnight to precipitate 1-*O*-(2,2,2-trichloroethoxycarbonyl)-1,5-quinide as a white amorphous powder, which was removed by filtration and dried in vacuum.

Methyl chloroformate (9.4 mL) was added dropwise at 0 °C to a solution of caffeic acid (51 mmol) in aqueous NaOH (1 mol/L, 150 mL) and was, then, stirred for 20 min at 0 °C to afford a yellow residue. After neutralization of the reaction mixture with aqueous HCl (2 mol/L), the residue was separated by filtration. Recrystallization from aqueous ethanol (50%, 250 mL) afforded 3,4-*O*-dimethoxycarbonyl caffeic acid as white crystals. Thionyl chloride (96.0 mmol/L) was added dropwise to 3,4-*O*-dimethoxycarbonyl caffeic acid (14.0 mmol), the suspension heated to 90 °C until gas development was finished, and residual thionyl chloride removed with a stream of nitrogen gas. Recrystallization with toluene (150 mL) afforded 3,4-*O*-dimethoxycarbonyl caffeic acid chloride as yellowish crystals.

A solution of 3,4-*O*-dimethoxycarbonyl caffeic acid chloride (14.0 mmol) in dichloromethane (30 mL) was added dropwise to a solution of 1-*O*-(2,2,2-trichloroethoxycarbonyl)-γ-quinide (4.21 mmol) and pyridine (17.9 mmol) in dichloromethane (30 mL) at 0 °C. After the mixture had been stirred overnight, the solvent was removed in vacuum, and the residue was dissolved in ethyl acetate (200 mL) and, then, sequentially extracted with an aqueous HCl solution (1 mol/L, 35 mL), an aqueous $NaHCO_3$ solution (2%, 35 mL), and water (35 mL). After the organic layer had been dried over Na_2SO_4, the solvent was separated in vacuum to afford pure 1-*O*-(2,2,2-trichloroethoxycarbonyl)-3,4-bis[3,4-*O*-(dimethoxycarbonyl)caffeoyl]-1,5-quinide. An aliquot (2.21 mmol) of this product was suspended with lithium chloride (23.6 mmol) in pyridine (20 mL) and then stirred for 7 days at 50 °C. After the solvent had been removed in vacuum, the residue was dissolved in ethyl acetate (100 mL), and the solution was sequentially extracted with an aqueous HCl solution (1 mol/L, 50 mL), an aqueous Na_2CO_3 solution (2%, 50 mL), and brine (50 mL). Drying of the organic layer over Na_2SO_4 and separation of the solvent in vacuum afforded a brown residue, which was applied on top of a glass column (300 mm × 30 mm) filled with a slurry of Polyamide MN-SC-6 material (Macherey-Nagel, Düren, Germany) conditioned in an ethyl acetate/methanol mixture (80/20, v/v). The column was rinsed with an ethyl acetate/methanol mixture (80/20, v/v) (500 mL), followed by ethyl acetate/methanol mixtures (300 mL each) with increasing amounts of methanol (from 30 to 100%). After separation of the solvent in vacuum, 70/30 (v/v) and 60/40 (v/v) ethyl acetate/methanol mixtures afforded the target compound 3,4-*O*-dicaffeoyl-γ-quinide, the identity of which was confirmed by comparison of chromatographic (RP-HPLC) and spectroscopic data (LC-MS/MS, NMR, and UV/vis) with those reported recently for 3,4-*O*-dicaffeoyl-γ-quinide isolated from thermally treated 3,4-*O*-dicaffeoyl γ-quinic acid (*9*).

3,4-O-Dicaffeoyl-γ-quinide (**9**) (the arbitrary numbering of the carbon atoms refers to the structure in **Figure 2**): UV/vis [5/5 (v/v) methanol/water] λ_{max} = 235, 300, 328 nm; LC-MS (ESI^-) *m*/*z* 497 (100; $[M-H]^-$); ^1H NMR (400 MHz, d_3-MeOD, COSY) δ 2.20−2.34 [m, 2H, H-C(2_{eq}), H-C(2_{ax})], 2.45−2.62 [m, 2H, H-C(6_{eq}), H-C(6_{ax})], 4.95 [dd, 1H, J = 5.6, 5.0 Hz, H-C(5_{eq})], 5.20 [ddd, 1H, J = 11.5, 6.6, 4.7 Hz, H-C(3_{ax})], 5.63 [dd, 1H, J = 4.7, 5.0 Hz, H-C(4_{eq})], 6.16 [d, 1H, J = 15.9 Hz, H-C(5′)], 6.40 [d, 1H, J = 15.7 Hz, H-C(5″)], 6.70 [d, 1H, J = 8.1 Hz, H-C(3′)], 6.81 [d, 1H, J = 8.2 Hz, H-C(3″)], 6.83 [dd, 1H, J = 8.1, 2.1 Hz, H-C(2′)], 6.99 [dd, 1H, J = 8.2, 2.1 Hz, H-C(2″)], 7.02 [d, 1H, J = 2.1 Hz, H-C(1′)], 7.10 [d, 1H,

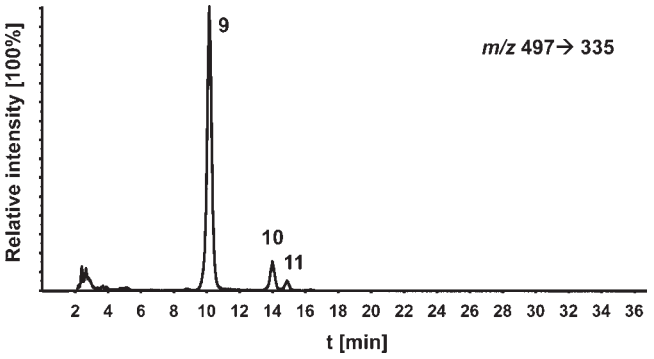

Figure 3. HPLC-MS/MS analysis of the dicaffeoyl quinides **9**−**11** in a freshly prepared coffee brew using the multiple-reaction monitoring mode.

J = 2.1 Hz, H-C(1″)], 7.50 [d, 1H, J = 15.9 Hz, H-C(4′)], 7.64 [d, 1H, J = 15.7 Hz, H-C(4″)].

Quantitative Analysis of *O*-Caffeoyl Quinic Acids 1−3 and *O*-Caffeoyl-Quinides 4−8. Aliquots (10 μL) of standard coffee beverages were analyzed by means of HPLC-UV/vis on a Phenyl-hexyl Luna, 250 mm × 4.6 mm (inside diameter), 5 μm column (Phenomenex) using a gradient of methanol (solvent A) and aqueous ammonium formate buffer (0.25 mol/L, pH 3.5, solvent B) as the mobile phase. Chromatography was performed at a flow rate of 0.8 mL/min starting with 25% solvent A, and then the content of solvent A was increased to 28% within 38 min and then to 100% within 6.5 min and, finally, kept at 100% solvent A for an additional 5 min. After identification of 3-*O*-caffeoyl quinic acid (**1**), 5-*O*-caffeoyl quinic acid (**2**), 4-*O*-caffeoyl quinic acid (**3**), 5-*O*-caffeoyl-*muco*-γ-quinide (**4**), 3-*O*-caffeoyl-γ-quinide (**5**), 4-*O*-caffeoyl-*muco*-γ-quinide (**6**), 5-*O*-caffeoyl-*epi*-δ-quinide (**7**), and 4-*O*-caffeoyl-γ-quinide (**8**) upon comparison of chromatographic (retention time) and spectroscopic (UV/vis and LC-MS/MS) data with those obtained for the reference compounds, quantification was performed by comparing the peak area obtained at 324 nm with those of defined standard solutions of 5-*O*-caffeoyl quinic acid in a 1/1 (v/v) methanol/water mixture (pH 3.5).

Quantitative Analysis of *O*-Dicaffeoyl-Quinides 9−11. Aliquots (10 μL) of standard coffee beverages were analyzed by means of LC-MS/MS operated in the negative ionization mode on a RP-18 Synergi Fusion, 150 mm × 2.0 mm (inside diameter), 5 μm column (Phenomenex) using a gradient of methanol (solvent A) and 0.1% aqueous formic acid (solvent B) at a flow rate of 0.25 mL/min. We performed chromatography by increasing the content of solvent A from 40 to 60% in 15 min and then increasing the content of solvent A to 100% within 25 min and, finally, keeping the level at 100% solvent A for an additional 5 min. After identification of 3,4-*O*-dicaffeoyl-γ-quinide (**9**), 4,5-*O*-dicaffeoyl-*muco*-γ-quinide (**10**), and 3,5-*O*-dicaffeoyl-*epi*-δ-quinide (**11**) upon comparison of chromatographic (retention time) and spectroscopic (LC-MS/MS) data with those obtained for the reference compounds, we performed quantification by comparing the peak area obtained for the trace of the corresponding mass transition with those of defined standard solutions of synthetic 3,4-dicaffeoyl-γ-quinide dissolved in a mixture of methanol and 0.1% aqueous formic acid (1/1, v/v). Using the multiple-reaction monitoring (MRM) mode, the target quinides **9** (m/z 497.0 → 335.0), **10** (m/z 497.0 → 335.0), and **11** (m/z 497.0 → 335.0) were analyzed using the mass transitions given in parentheses (**Figure 3**).

Quantitative Analysis of 4-Vinylcatechol Oligomers. Aliquots (900 μL) of standard coffee beverages were acidified to pH 2 with hydrochloric acid (50 μL) and spiked with a solution of the internal standard resveratrol (50 μL, 2.5 μg/mL) in a 1/1 (v/v) methanol/water mixture (pH 3.5). After homogenization of the samples, aliquots (20 μL) were analyzed by means of HPLC-MS/MS operated in the negative ionization mode on a Zorbax Eclipse XDB C18, 150 mm × 2.1 mm (inside diameter), 5 μm column (Agilent, Waldbronn, Germany) using the MRM mode with the following mass transitions given in parentheses: resveratrol (m/z 227.0 → 143.1), **12** (m/z 271.0 → 109.0), **13** (m/z 271.0 → 109.0), **14** (m/z 271.1 → 109.0), **15** (m/z 273.2 → 109.0), **16** (m/z 271.1 → 109.1), **17** (m/z 271.1 → 109.1), **18** (m/z 407.1 → 297.2), **19** (m/z 407.1 → 297.2), and **20** (m/z 407.2 → 161.2). Chromatography was performed with a gradient of acetonitrile (solvent A) and 0.1% aqueous formic acid (solvent B) at a flow rate of 0.25 mL/min starting with 25% solvent A, and the content of solvent A was increased to 28% within 5 min, then to 30% within 20 min, and then to 100% within 5 min and, finally, kept at 100% for an additional 2 min. The target compounds **12**−**20** were quantified via resveratrol as the internal standard. The concentrations of the individual 4-vinylcatechol oligomers were calculated using calibration curves prepared by plotting peak area ratios of analyte to internal standard against concentration ratios (0.3−3) of each analyte to the internal standard using linear regression.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (Agilent) consisted of a series HP 1050-type quaternary pump system, an HP 79855 A/B-type autosampler, and a MWD 79854 A-type multiwavelength detector.

High-Performance Liquid Chromatography with Tandem Mass Spectrometry (HPLC-MS/MS). The Agilent 1100 Series HPLC system consisted of a pump, a degasser, and an autosampler (Agilent) and was connected to a 4000 Q Trap triple quadrupole/linear ion trap mass spectrometer (Applied Biosystems/MDS Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) device running in negative ionization mode with a spray voltage of −4500 V. Nitrogen served as the curtain (20 psi), nebulizer (35 psi), and heater (45 psi) gas. Detection was performed in the multiple-reaction monitoring (MRM) mode, recording the transition from the negative pseudomolecular ion $[M - H]^-$ to the main fragment generated after collision-induced dissociation. The quadrupoles operated at unit mass resolution. Sciex Analyst (version 1.4.1) was used for instrumentation control and data analysis.

Sensory Analyses. *General Conditions and Panel Training*. Twelve assessors (five male and seven female, 22−40 years of age), who gave the informed consent to participate in the sensory tests of this investigation and who had no history of known taste disorders, were trained in weekly training sessions for at least two years as described previously (*18*−*21*) and were, therefore, familiar with the techniques applied. The sensory analyses were performed at 22 °C in an air-conditioned room with separated booths in three independent sessions. To prevent cross-modal interactions with olfactory responses, the panellists used nose clips.

Bitter Taste Analysis. Freshly prepared coffee beverages were used for sensory analysis. The samples (5 mL) were presented to the sensory panelists who were asked to score the bitter taste intensity and bitter quality on a scale from 0 (not detectable) to 10.0 (strong taste impression).

RESULTS AND DISCUSSION

Recent investigations led to the discovery of the monocaffeoyl quinic acid lactones **4**−**8** (**Figure 1**), the dicaffeoyl quinic acid lactones **9**−**11**, the 4-vinylcatechol dimers **12**−**17**, and the 4-vinylcatechol trimers **18**−**20**, all of which are generated upon thermal degradation of 5-*O*-caffeoyl quinic acid (**2**), as intense bitter compounds in roasted coffee (*9*, *10*). To investigate the influence of the roasting parameters on the development of bitter taste as well as the generation of these bitter compounds in roasted coffee, first, analytical procedures were developed for the quantitative analysis of the bitter tastants **4**−**20** as well as their parent monocaffeoyl quinic acids **1**−**3** in coffee brews.

Quantitative Analysis of Compounds 1−20 in Coffee Brew. For the quantitative analysis of monocaffeoyl quinic acids (**1**−**3**) and monocaffeoyl quinides (**4**−**8**), coffee brews, prepared by percolating samples of freshly ground roasted coffee with hot tap water, were directly analyzed by means of HPLC-UV/vis using Phenyl-hexyl material as the stationary phase. As preliminary studies revealed the same extinction coefficients for monocaffeoyl quinic acids and the corresponding quinides, 5-*O*-caffeoyl quinic acid was used as the external standard for quantification of compounds **1**−**8**.

Analysis of the dicaffeoyl-quinides 3,4-*O*-dicaffeoyl-γ-quinide (**9**), 4,5-*O*-dicaffeoyl-*muco*-γ-quinide (**10**), and 3,5-*O*-dicaffeoyl-*epi*-δ-quinide (**11**) in coffee brew was successfully achieved by means of RP18-HPLC-MS/MS running in the multiple-reaction monitoring (MRM) mode (**Figure 3**). Quantitative determination of bitter compounds **9**−**11** was performed by comparing the peak

area obtained for the trace of the corresponding mass transition with those of defined standard solutions of purified 3,4-*O*-dicaffeoyl-γ-quinide prepared from quinic acid and caffeic acid in the multistep synthesis given in **Figure 2**.

As preliminary studies demonstrated that the 4-vinylcatechol oligomers **12**−**20** are rather unstable at the pH value of coffee beverages (pH 4.9−6.5) and comparatively stable under more acidic conditions, aliquots of the freshly prepared coffee brews were acidified to pH 2.0 with aqueous hydrochloric acid and then directly injected into the RP18-HPLC-MS/MS system for monitoring of the mass transitions for the 4-vinylcatechol oligomers **12**−**20** (**Figure 4**). For quantitative analysis, resveratrol showing stability and ionization behavior similar to those of the target analytes was used as the internal standard.

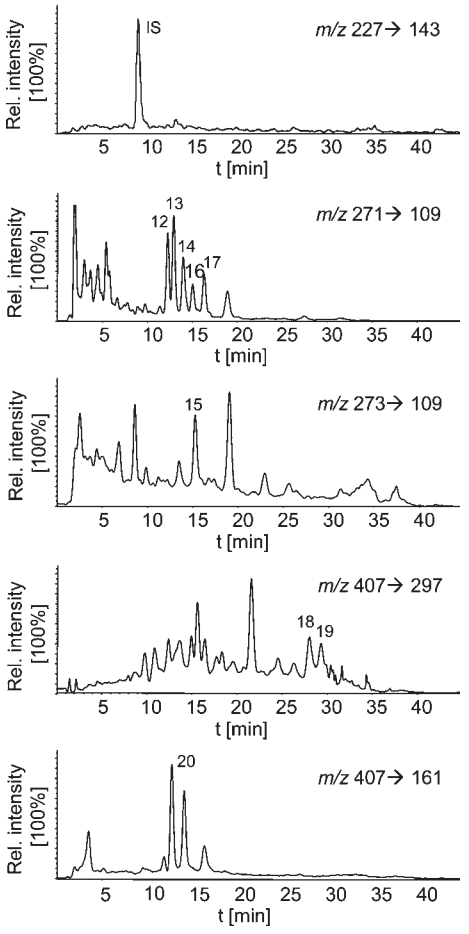

Figure 4. HPLC-MS/MS analysis of the 4-vinylcatechol oligomers **12**−**20** in a freshly prepared coffee brew using the multiple-reaction monitoring mode.

Influence of the Roast Gas Temperature on the Bitterness and Concentration of 1−20 in Coffee Brew. To investigate the influence of the roast gas temperature on the formation of bitter taste compounds during coffee bean roasting, coffee brews were freshly prepared from ground coffee beans roasted for a constant time of 240 s at 190−280 °C.

First, the bitter taste intensity of these coffee beverages was sensorially evaluated on a scale from 0 (not detectable) to 10 (intense bitterness) by a trained sensory panel. These studies showed that the coffee samples roasted for 240 s at 190−210 °C did not impart any bitterness (**Table 1**). Increasing the roast gas temperature from 220 to 280 °C induced a strong increase in the perceived bitterness of the coffee brews from 0.25 to 8.0 (**Table 1**). Most interestingly, not only an increase in the taste intensity but also a change in the quality of the bitter taste was observed depending on the roast gas temperature applied. The pleasant, typical coffeelike bitterness of the more mildly roasted coffees changed to a harsh, burnt, long-lasting, and espresso-like bitter taste modality perceived in particular at the posterior tongue and the throat when gas temperatures above 240 °C were applied for coffee roasting.

To investigate the question of which of the bitter compounds might contribute to the change in bitter taste profile observed with increasing roast gas temperature, we quantitatively assessed the bitter compounds **4**−**20** as well as the parent monocaffeoyl quinic acids (**1**−**3**) in the coffee brew prepared from each coffee sample by means of HPLC-UV/vis (**1**−**8**) and HPLC-MS/MS (**9**−**20**) (**Figure 5**). The quantitative studies revealed the nonbitter 5-*O*-caffeoyl quinic acid (**1**), followed by 4-*O*-caffeoyl (**2**) and 3-*O*-caffeoyl quinic acid (**3**), as the predominant polyphenols in coffee roasted for 240 s at 190 °C (**Figure 5A**). In comparison, only trace amounts of bitter compounds **4**−**20** were detectable (**Figure 5B**−**D**), thus being well in line with the lacking bitterness in that coffee sample (**Table 1**).

Increasing the roast gas temperature induced a rapid degradation of the monocaffeoyl quinic acids; e.g., increasing the temperature from 190 to 280 °C decreased the concentration of the sum of caffeoyl quinic acids **1**−**3** from 2274.8 to 33.0 mg/L of coffee brew. Running in parallel to the degradation of caffeoyl quinic acids, the concentration of the monocaffeoyl-quinides **4**−**8** increased with increasing roasting temperature and went through a maximum at 240 °C; e.g., the total amount of the bitter monocaffeoyl-quinides **4**−**8** increased 10-fold from 17.5 to 174.5 mg/L when the roast gas temperature was increased from 190 to 240 °C. Among the monocaffeoyl-quinides, 3-*O*-caffeoyl-γ-quinide (**5**) and 4-*O*-caffeoyl-γ-quinide (**8**) were found as the quantitatively predominating isomers, whereas the levels of 5-*O*-caffeoyl-*muco*-γ-quinide (**4**), 4-*O*-caffeoyl-*muco*-γ-quinide (**6**),

Table 1. Intensity and Quality of the Bitterness Perceived in Standard Coffee Brews Made from Ground Coffee Beans Roasted for 240 s at 190−280 °C

roast gas temperature (°C)	bitter intensity[a]	predominating bitter quality
190	0	nd[b]
200	0	nd[b]
210	0	nd[b]
220	0.25	mild, pleasant, typical coffeelike bitterness
230	0.75	mild, pleasant, typical coffeelike bitterness
240	1.5	coffeelike bitterness
250	2.5	harsh, espresso-like bitterness perceived in the back of the throat
260	4.0	harsh, espresso-like bitterness perceived in the back of the throat
270	6.5	long-lasting, harsh, espresso-like bitterness perceived in the back of the throat
280	8.0	long-lasting, strongly harsh bitterness perceived in the back of the throat

[a] Bitter intensities of the standard coffee beverages (54 g/L) were evaluated on a scale from 0 (not detectable) to 10.0 (strong taste impression). [b] Not detectable.

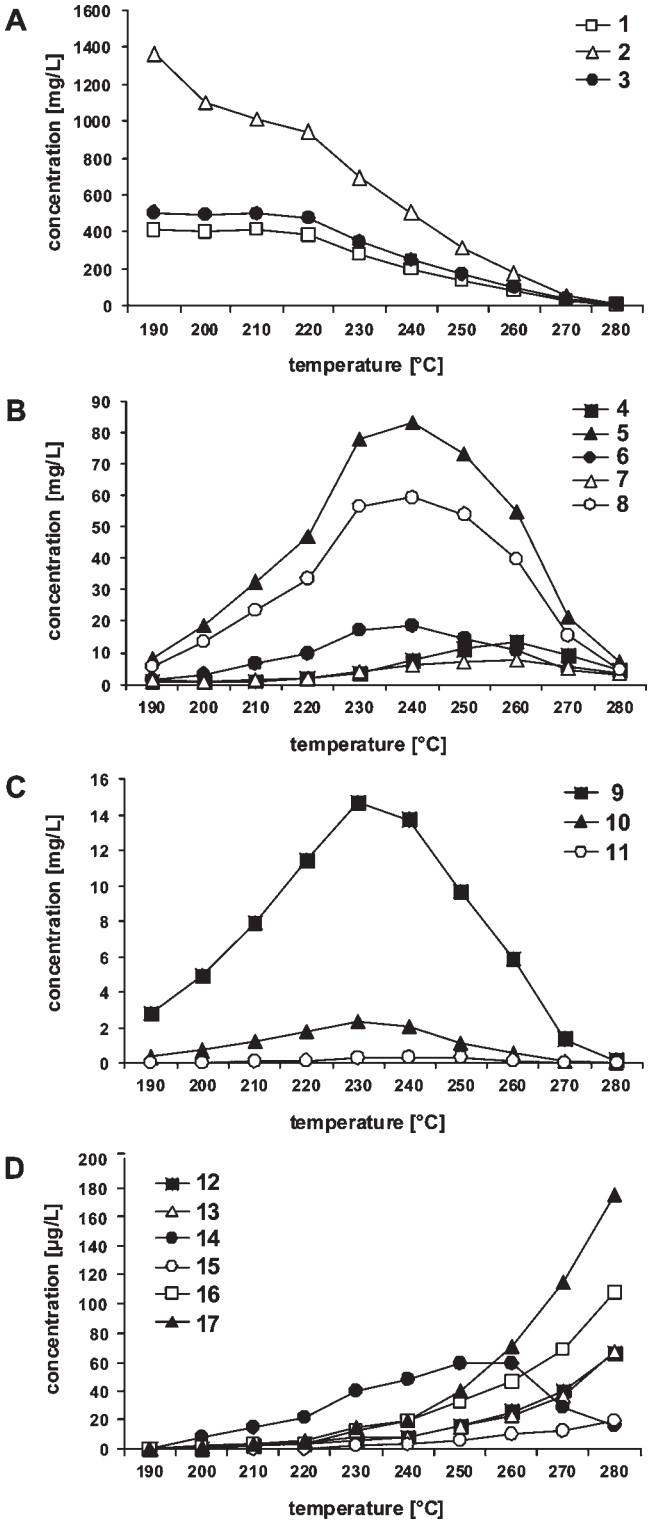

Figure 5. Influence of the roast gas temperature on the concentrations of the monocaffeoyl quinic acids **1**−**3** (**A**), the bitter-tasting monocaffeoyl-quinides **4**−**8** (**B**), the bitter-tasting dicaffeoyl-quinides **9**−**11** (**C**), and the lingering, harsh bitter-tasting 4-vinylcatechol oligomers **12**−**17** (**D**) in coffee brews freshly prepared from Arabica coffee beans roasted for 240 s. Concentrations are given as means of triplicates (standard deviation of <15%).

and 5-*O*-caffeoyl-*epi*-δ-quinide (**7**) were substantially lower (**Figure 5B**). The transformation of the nonbitter monocaffeoyl quinic acids (**1**−**3**) into the monocaffeoyl-quinides, imparting a pleasant, coffee-specific bitter profile (*9*, *10*), is well in line with the increasing mild and pleasant bitterness of coffee samples roasted for 240 s at 220−240 °C (**Table 1**). A further increase of the roast gas temperature from 240 to 280 °C induced a decrease in the monocaffeoyl-quinide content; e.g., roasting at 280 °C generated the quinides **4**−**8** at rather low levels comparable to the amounts produced at 190 °C. These data clearly demonstrate the thermal instability of the pleasant bitter-tasting monocaffeoyl-quinides.

The quantitation of the concentration curve of the dicaffeoyl quinic acid lactones 3,4-*O*-dicaffeoyl-γ-quinide (**9**), 4,5-*O*-dicaffeoyl-*muco*-γ-quinide (**10**), and 3,5-*O*-dicaffeoyl-*epi*-δ-quinide (**11**) revealed a temperature dependency rather similar to that found for the monocaffeoyl-quinides **4**−**8** (**Figure 5C**). The 3,4-*O*-dicaffeoyl-γ-quinide (**9**) was found to be the major isomer accounting for more than 80% of the total amount of dicaffeoyl-quinides. At 190 °C, a total amount of only 3.2 mg/L was found for the dicaffeoyl-quinides, and after running through a mixmum of 17.4 mg/L at 230 °C, the bitter compounds **9**−**11** started to degrade again and were not detectable when roasting was performed at 280 °C.

In contrast to the caffeoyl-quinides **4**−**11**, the concentration of the 4-vinylcatechol oligomers **12**−**20** increased continuously with higher roast gas temperatures, running in parallel with the increasing harsh and espresso-like bitterness of the coffee samples roasted above 250 °C (**Figure 5D** and **Table 1**).

As an exception, the concentration of compound **14** went through a maximum at 250 °C and then decreased again, suggesting compound **14** as a precursor of the 4-vinylcatechol dimers **12**, **13**, **16**, and **17** and confirming the recently reported formation pathway showing the cyclization of **14** to give the hydroxylated phenylindanes **12**, **13**, **16**, and **17** (*10*). The concentrations of the derivatives **18**−**20** were at their detection thresholds and were therefore not quantitatively determined. Increasing the roast gas temperature from 190 to 280 °C increased the total concentration of 4-vinylcatechol oligomers from 0 to 452.6 μg/L of coffee brew.

Influence of the Roasting Time on the Concentration of Bitter Compounds 1−**20 in Coffee Brew.** In a second set of experiments, standard coffee brews were prepared from 10 coffee samples roasted at a constant temperature of 260 °C for 60−600 s and then analyzed for the bitter compounds **4**−**20** and the parent caffeoyl quinic acids **1**−**3** by means of HPLC-UV/vis and HPLC-MS/MS, respectively (**Figure 6**).

The caffeoyl quinic acids **1**−**3** were found to be rather unstable when the roasting time was increased; e.g., increasing the duration of roasting from 60 to 600 s decreased the total amount of caffeoyl quinic acids by a factor of 93 from 2350.2 to 25.4 mg/L of coffee brew (**Figure 6A**). Already after 240 s, the major part of the caffeoyl quinic acids were degraded and only 359.1 mg/L was present in the coffee brew.

Running in parallel to the decrease in the content of caffeoyl quinic acids, the formation of the bitter-tasting mono- and dicaffeoyl-quinides was favored when the roasting time was increased from 60 to 180 s; e.g., the amounts of caffeoyl-quinides (**4**−**8**) and dicaffeoyl-quinides (**9**−**11**) increased by factors of 7 and 4, respectively (**Figure 6B,C**). After passing the maximum, the caffeoyl quinide concentration diminished rapidly; e.g., elongation of the roasting time from 180 to 600 s diminished the concentration of the monocaffeoyl-quinides from 181.5 to 16.9 mg/L and that of dicaffeoyl-quinides from 5.69 to 0.10 mg/L.

Quantitative analysis of the 4-vinylcatechol oligomers revealed a rapid increase in the concentration of the compound **14** to reach a maximum level of 70 μg/L after roasting for 240 s (**Figure 6D**). In comparison, the formation of compounds **12**, **13**, **16**, and **17** was favored when the beans were roasted for more than 180 s at 260 °C; e.g., the total amount of these 4-vinylcatechol dimers

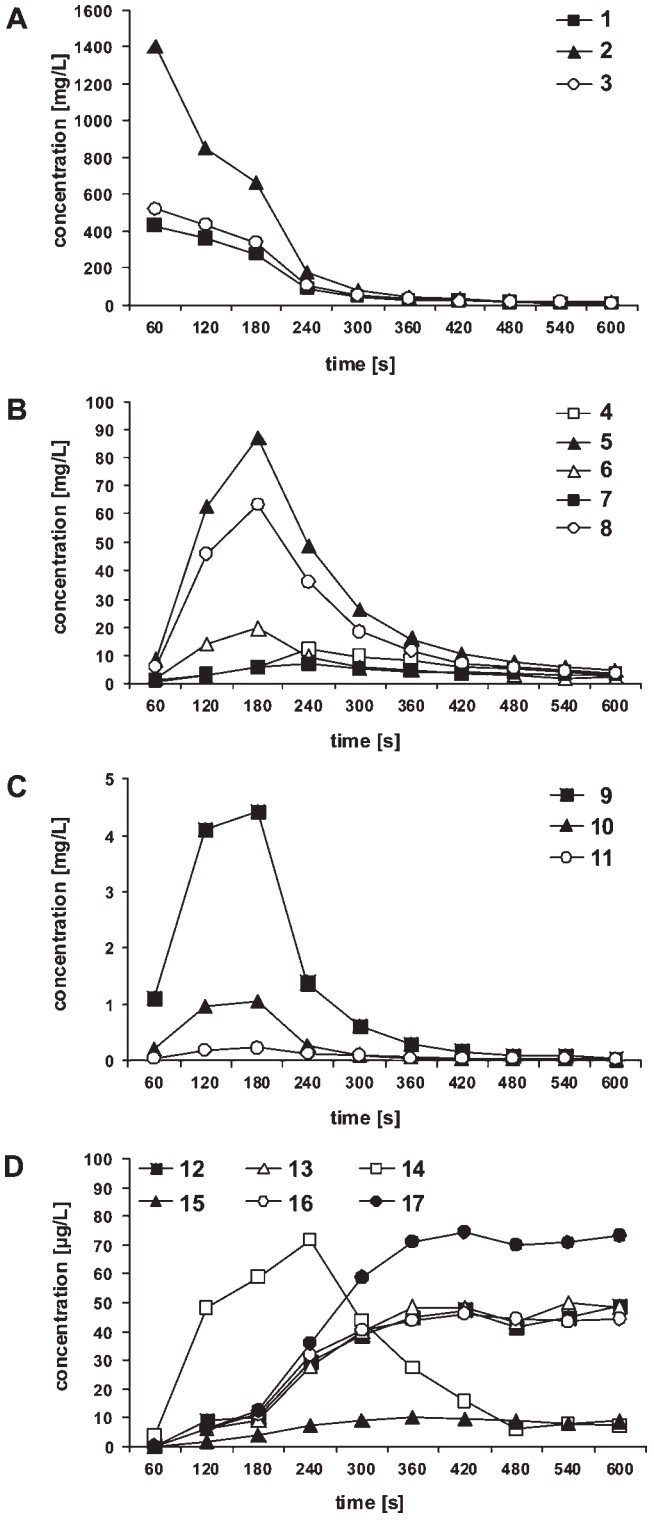

Figure 6. Influence of the roasting time on the concentrations of the monocaffeoyl quinic acids **1**−**3** (**A**), the bitter-tasting monocaffeoyl-quinides **4**−**8** (**B**), the bitter-tasting dicaffeoyl-quinides **9**−**11** (**C**), and the lingering, harsh bitter-tasting 4-vinylcatechol oligomers **12**−**17** (**D**) in coffee brews freshly prepared from Arabica coffee beans roasted at 260 °C. Concentrations are given as means of triplicates (standard deviation of <15%).

increased from 3.5 to 245.3 μg/L after 600 s. Again, these data are well in line with the proposed formation pathway of the phenylindanes **12**, **13**, **16**, and **17** running via compound **14** as a key intermediate (*10*). Independent of the roasting degree, the levels of compound **15** were rather low, and the trimers **18**−**20** were detected only in trace amounts (data not shown).

The data obtained clearly demonstrate for the first time that the composition of bitter-tasting compounds in coffee is strongly dependent on the roasting time and gas temperature applied during coffee roasting. The monocaffeoyl- and dicaffeoyl-quinides, exhibiting a pleasant coffee-typical bitter taste profile, were found to be preferentially formed under slight to medium roasting degrees and were observed to be degraded again after running through a maximum, thus confirming earlier literature findings (*12*−*16*). In comparison, the formation of the long-lasting and harsh bitter-tasting 4-vinylcatechol oligomers was found to be favored under more severe roasting conditions. The molecular changes among the group of the bitter tastants (**4**−**17**) seem to be well in line with the influence of the roasting degree on the bitter taste quality perceived for the corresponding coffee beverages; e.g., slight to medium roasted coffee gives a mild, pleasant, and coffeelike bitter quality, whereas more severe roasting was found to result in a lingering, harsh bitter beverage.

Release of Monocaffeoyl Quinic Acids and Bitter Tastants from Ground Coffee during Sequential Hot Water Extraction. To gain the first insight into the release of selected taste compounds from ground coffee upon water percolation, we performed a model experiment by placing freshly ground coffee into a cartridge and successively percolating the coffee with 10 equal 10 mL volumes of boiling water. The effluent of each fraction was collected in ice-cooled volumetric flasks and then used for the quantitative analysis of the monocaffeoyl quinic acids **1**−**3** and the bitter compounds **4**−**17** by means of HPLC-UV/vis and HPLC-MS/MS. In an additional experiment, the same amount of coffee powder was placed in a cartridge and percolated with the total amount of water volume in only a single elution step.

The quantitative analysis of caffeoyl quinic acids revealed a rapid decrease of the concentration of **1**−**3** within the first 30 mL of water; e.g., the total amount of caffeoyl quinic acids in the first 10 mL aliquot was 40 mg, accounting for 56% of the total amount of caffeoyl quinic acids, followed by 18 mg in the second 10 mL aliquot used for percolation (**Figure 7A**). The later fractions contained comparatively small amounts of caffeoyl quinic acids; e.g., <1.0 mg of **1**−**3** was present in the sixth aliquot (fraction of 51−60 mL), thus demonstrating that the caffeoyl quinic acids were rapidly extracted from the coffee powder by hot water percolation.

The release patterns of the bitter-tasting monocaffeoyl quinides **4**−**8** were found to be rather similar to that found for the quinic acids **1**−**3**, although the elution curve was slightly more flat when compared to that of the caffeoyl quinic acids which is to be expected due to the somewhat weaker polarity of the lactones (**Figure 7B**). The first 10 mL volume contained 37% of the total amount of extracted quinides **4**−**8**. In comparison, the concentrations of the dicaffeoyl-quinides accounted only for 18% of the total amount of **9**−**11**, thus giving strong evidence that the less polar dicaffeoyl quinic acid lactones were extracted rather slowly from the coffee powder (**Figure 7C**). The dicaffeoyl-quinide content in the last 10 mL volume used for percolation (91−100 mL) was still 12 μg, accounting for 35% of the concentration of the first sample, thus demonstrating a rather less effective release of this class of bitter lactones from ground coffee.

Quantitative analysis of the harsh bitter-tasting 4-vinylcatechol oligomers **12**−**17** revealed a different release behavior when compared to that of the lactones (**Figure 7D**). Interestingly, the amounts of compounds **12**−**17** per fraction obtained increased within the first 40 mL of percolation and reached a maximum of 2.5 μg in the fraction of 31−40 mL. Thereafter, the 4-vinylcatechol oligomer content per fraction decreased again slightly to

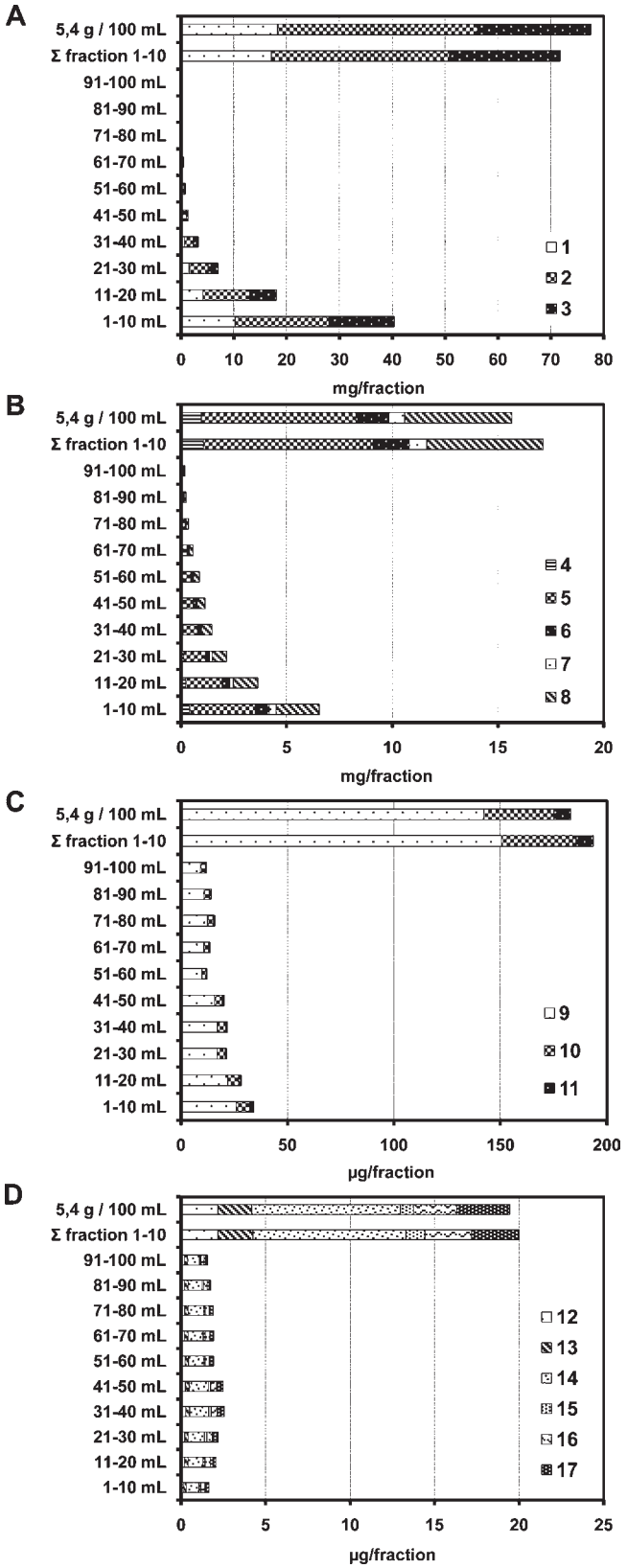

Figure 7. Release of monocaffeoyl quinic acids **1**−**3** (**A**), the bitter-tasting monocaffeoyl quinides **4**−**8** (**B**), the bitter-tasting dicaffeoyl quinides **9**−**11** (**C**), and the lingering, harsh bitter-tasting 4-vinylcatechol oligomers **12**−**17** (**D**) from coffee powder upon percolation with hot water. Concentrations are given as means of triplicate determinations (standard deviation of <10%).

~1.6 μg in the last 10 mL used for percolation, still accounting for 95% of the content found in the first filtrate. These data clearly indicate that the 4-vinylcatechol derivatives were released rather slowly from the coffee powder when compared to the lactones and seem to run in parallel with the polarity of the target compounds.

The data obtained in these studies imply that the targeted generation of single groups of bitter-tasting compounds by a knowledge-based control of the roasting conditions and/or the differential release of the bitter key compounds from coffee powder via the careful control of the extraction conditions might be helpful tools for tailoring the bitter taste profile of coffee beverages.

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Received for review December 17, 2009. Revised manuscript received February 11, 2010. Accepted February 12, 2010.