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Quantitative Precursor Studies on Di- and Trihydroxybenzene Formation during Coffee Roasting Using "In Bean" Model Experiments and Stable Isotope Dilution Analysis

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The objective of this study was to investigate the potential of various raw bean components as precursors of pyrogallol (**1**), hydroxyhydroquinone (**2**), catechol (**3**), 4-ethylcatechol (**4**), 4-methylcatechol (**5**), and 3-methylcatechol (**6**) under quasi "natural" roasting conditions by using the recently developed "in bean" model roast experiments. Freeze-dried, fully extracted bean shells were loaded with aqueous solutions of either single coffee compounds or fractions isolated from the raw bean solubles. After freeze-drying, these reconstituted beans were roasted, aqueous coffee brews were prepared, and the target phenols were quantified by means of a stable isotope dilution assay with LC-MS/MS detection. On the basis of the quantitative data, it can be concluded that upon coffee bean roasting, catechol (**3**) is primarily formed by degradation of caffeoylquinic acids from both the caffeic acid and the quinic acid moiety of the molecule, as well as from Maillard-type reactions from carbohydrates and amino acids. In contrast, pyrogallol (**1**) and hydroxyhydroquinone (**2**) are efficiently generated from carbohydrates and amino acids and, in addition, from free or chlorogenic acid bound quinic acid moieties. 4-Ethylcatechol (**4**) is exclusively generated upon thermal breakdown of caffeic acid moieties. 3-Methylcatechol (**6**) is formed primarily from the Maillard reactions and, to a minor extent, also from various phenolic precursors, whereas 4-methylcatechol (**5**) is produced in trace amounts only from all of the different precursors investigated. On the basis of this precursor study, reaction routes explaining the formation of the target phenols are proposed.

KEYWORDS: Coffee; chlorogenic acids; caffeic acid; quinic acid; pyrogallol; hydroxyhydroquinone; catechol; 4-ethylcatechol; 4-methylcatechol; 3-methylcatechol; stable isotope dilution analysis

INTRODUCTION

In addition to its stimulatory effect, the wide popularity of a freshly brewed coffee beverage is mainly based on its alluring aroma. Unfortunately, the freshness of a coffee brew cannot be preserved because the desirable aroma, in particular, the roasty−sulfury note is rather unstable and changes shortly after preparation of the coffee brew (*1*−*4*). Systematic studies based on GC−olfactometric techniques as well as quantitative studies revealed that the concentrations of odor-active thiols such as 3-methyl-2-butene-1-thiol, 3-mercapto-3-methylbutyl formate, 2-methyl-3-furanthiol, methanethiol, and, in particular, 2-furfurylthiol, one of the coffee character-impact odorants, are drastically reduced upon coffee storage (*1*−*4*) as well as additional processing such as manufacturing of instant coffee (*5*) or sterilization of ready-to-drink canned coffee beverages (*6*).

Systematic studies investigating the molecular basis for the storage-induced decrease of the thiol concentration in coffee demonstrated that the dark-colored coffee melanoidins exhibiting molecular masses above 3000 Da are able to effectively trap 2-furfurylthiol by covalent binding (*2*, *3*). Furthermore, a series of model experiments identified 1,4-alkylpyrazinium radical cations, known as important intermediates in roasting-induced melanoidin genesis, as covalently binding 2-furfurylthiol (*2*, *3*).

To perform coffee model experiments under more realistic conditions, we recently used so-called biomimetic "in bean" experiments to clarify the precursors of thiol receptive components in raw coffee beans (*7*). To achieve this, intact raw beans were exhaustively extracted with hot water to obtain the water extractables and the nonsoluble, intact bean shell, which can be used as the "matrix" or "architecture" in model roasting experiments using a batch roaster. This allowed us to infuse the freeze-dried bean shell with aqueous solutions of either single coffee compounds or fractions isolated from the raw bean solubles, to roast these reconstituted beans after freeze-drying, and finally to measure the activity of these compounds "in bean" as precursors of thiol binding sites (*7*). Using this technology, chlorogenic acids such as the quantitatively predominating 5-*O*-caffeoylquinic acid (*8*, *9*), as well as their thermal degradation products caffeic acid and quinic acid, were unequivocally identified as low molecular weight precursors for thiol-binding

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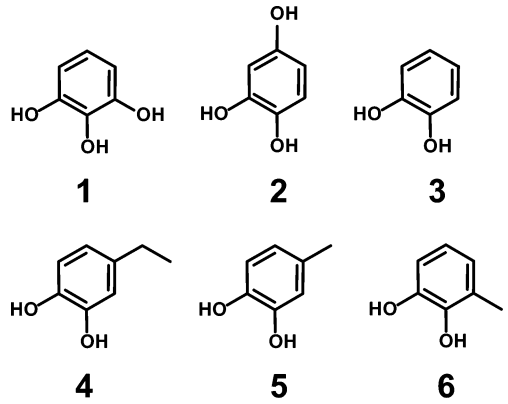

Figure 1. Chemical structures of pyrogallol (**1**), hydroxyhydroquinone (**2**), catechol (**3**), 4-ethylcatechol (**4**), 4-methylcatechol (**5**), and 3-methylcatechol (**6**).

receptors generated upon roasting (*7*). It has been known for a long time that chlorogenic acids are degraded during coffee roasting to give various reaction products such as the bitter-tasting chlorogenic acid lactones (*10*), caffeic acid, quinic acid and quinic acid lactones (*11*), and also a variety of di- and trihydroxyphenols liberated from the further degradation of caffeic acid and quinic acid (*12*, *13*). Among these di- and trihydroxybenzenes, pyrogallol (**1**; **Figure 1**), hydroxyhydroquinone (**2**), catechol (**3**), 4-ethylcatechol (**4**), 4-methylcatechol (**5**), and 3-methylcatechol (**6**) have been reported as the predominant candidates (*12*, *14*).

Catechol (**3**), first identified by Reichstein and Staudinger (*15*) and later confirmed by Rahn and König (*16*) as a constituent of roast coffee infusions, is reported as the most predominant dihydroxyphenol in roasted coffee (*12*, *16*, *17*) and was shown to originate from the degradation of caffeic acid (*12*, *13*, *18*) and quinic acid (*12*, *13*), both liberated from chlorogenic acids upon hydrolysis. In comparison, 4-ethylcatechol (**4**), first identified by Rahn and König (*16*), is believed to be exclusively generated from caffeic acid hydrolytically liberated from chlorogenic acids (*13*, *19*). Both of the trihydroxybenzenes, pyrogallol (**1**) and hydroxyhydroquinone (**2**), are reported to be formed upon pyrolysis of quinic acid (*12*, *16*, *18*), but for none of these compounds are reliable quantitative data available on the precursor potential of individual coffee components. Although 4-methylcatechol (**5**) and 3-methylcatechol (**6**) were identified in roasted coffee more than 25 years ago (*12*, *16*), their formation pathways are still unclear.

As heating potential precursors in a laboratory oven are not suitable to mimic the real conditions existing in a single coffee bean, which are agreed to perform as a kind of miniaturized pressure reactor unit, the recently developed "in bean" model experiments (*7*) combined with stable isotope dilution analysis developed recently for di- and trihydroxybenzene quantitation (*20*) would open the possibility to reliably measure the precursor potential of individual raw coffee constituents for the formation of phenols **1**−**6**. The purpose of this study was, therefore, to investigate the potential of various raw bean components as precursors of the di- and trihydroxybenzenes **1**−**6** by using promising "in bean" model roast experiments.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: pyrogallol (**1**), hydroxyhydroquinone (**2**), catechol (**3**), 4-methylcatechol (**5**), 3-methylcatechol (**6**), caffeic acid, ferulic acid, 5-caffeoylquinic acid (Sigma-Aldrich, Steinheim, Germany); quinic acid, L-glutamic acid, L-proline, L-alanine, L-asparagine, 4-aminobutyric acid, L-aspartic acid, sucrose, galactose, glucose, fructose, formic acid, ammonium sulfate (Merck KGA, Darmstadt, Germany); and 4-ethylcatechol (**4**) (Lancaster, Eastgate, U.K.). Organic solvents were of HPLC grade, and water was of Millipore grade. The labeled internal standards d_3-pyrogallol (***d*$_3$-1**), d_4-catechol (***d*$_4$-3**), d_3-4-ethylcatechol (***d*$_3$-4**), d_3-4-methylcatechol (***d*$_3$-5**), and d_3-3-methylcatechol (***d*$_3$-6**) were synthesized following the procedure developed recently (*13*). The total chlorogenic acid (tCA) extract was isolated from raw coffee beans (Arabica, Colombia) as described recently (*7*, *21*) .

Table 1. Amounts of Precursor Compounds Used for Infusion into 50 g of Raw Bean Solids

expt	precursor	amount (g)
A	bean extract (BE)	12.04
B	tCA fraction	3.03
C	5-*O*-caffeoylquinic acid	3.03
D	caffeic acid	1.54
E	ferulic acid	1.66
F	quinic acid	1.65
G	carbohydrates + amino acids	
	sucrose	3.97
	galactose	0.01
	glucose	0.04
	fructose	0.04
	L-glutamic acid	0.06
	L-proline	0.02
	L-alanine	0.02
	L-asparagine	0.02
	4-aminobutyric acid	0.02
	L-aspartic acid	0.01

Total Extraction of Raw Coffee Beans. Following a procedure reported in the literature (*7*, *22*), whole green coffee beans (Arabica, Colombia, 50 kg) were extracted with demineralized hot water at 95 °C (1 × 60 min with 200 L, 3 × 30 min with 200 L). The water extracts obtained were combined and concentrated in a Luwa TFE evaporator at 40 °C (90 mbar) to achieve a total solid content of about 24%. This aqueous raw bean extract (BE) and the fully extracted bean shells (BS) were subsequently freeze-dried and stored in a desiccator at −18 °C until use.

Preparation of Coffee Bean Reconstitutes. Fraction BE (experiment A) and the tCA extract (experiment B) isolated from raw coffee beans were dissolved in water (25 mL) in concentrations corresponding to their natural concentrations in the authentic raw coffee bean (**Table 1**). In an additional experiment, the tCA extract was substituted by 5-*O*-caffeoylquinic acid (experiment C), which was dissolved in water (25 mL). Furthermore, caffeic acid (experiment D), ferulic acid (experiment E), and quinic acid (experiment F) were dissolved in water (25 mL) in amounts equimolar to the 5-*O*-caffeoylquinic acid (experiment C) (**Table 1**). Carbohydrates and amino acids (experiment G) were dissolved in water (25 mL) in concentrations corresponding to their natural concentrations in the authentic raw coffee bean (**Table 1**). All of these individual aqueous solutions were separately added to aliquots (50 g) of the freeze-dried, water-extracted raw bean shell (BS), slowly stirred overnight at room temperature, and then freeze-dried for 48 h.

Roasting and Grinding of Precursor-Loaded Coffee Beans. Prior to roasting, samples were freeze-dried for 48 h to adjust to the same humidity of 6.0%. The raw coffee beans (50 g) were roasted at 240 °C by means of a Probat BRZ II-type batch roaster (Emmerich, Germany) for 4.5 min. All roasted reconstitutes were ground by means of a batch mill (IKA, Staufen, Germany).

Preparation of Coffee Brews from Roasted Precursor-Loaded Coffee Beans. For the preparation of a coffee brew from the fully reconstituted coffee bean (BS + fraction BE), the powder obtained from the roast coffee (54 g) was percolated with hot water (1 L, 88−92 °C) using a drip filter (Kaffeefilterpapier no. 4) (ALDI Einkauf GmbH & Co. oHG, Essen, Germany). To account for the differences in weight of the individually reconstituted beans, the brews from powdered roasted extracted beans and reconstituted beans were prepared using the following amounts of ground powder for the individual

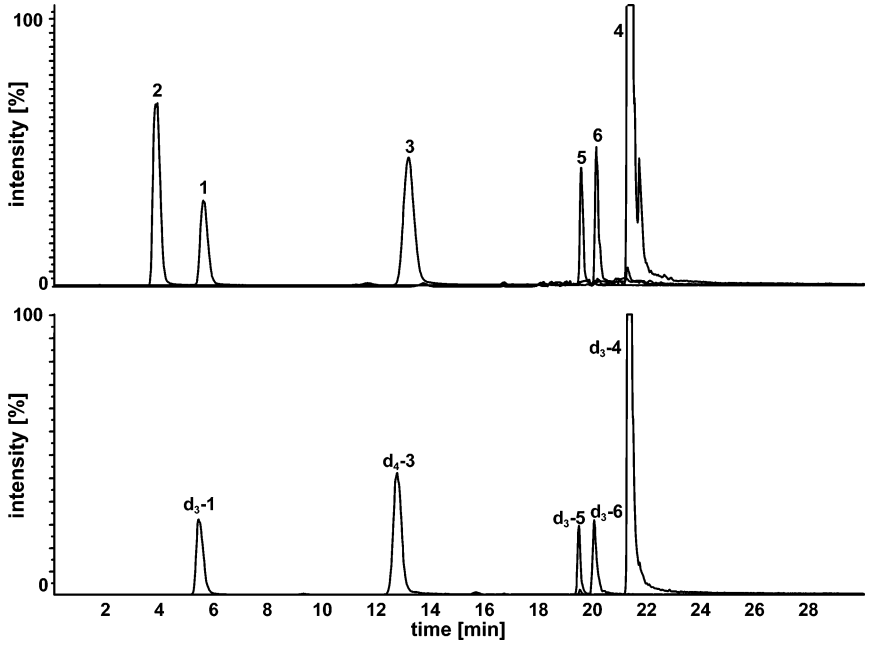

Figure 2. HPLC-MS/MS chromatogram of phenolic analytes and internal standards analyzed in a coffee brew: pyrogallol, **1**/d_3-**1**; hydroxyhydroquinone, **2**/d_3-**1**; catechol, **3**/d_4-**3**; 4-ethylcatechol, **4**/d_3-**4**; 4-methylcatechol, **5**/d_3-**5**; 3-methylcatechol, **6**/d_3-**6**.

experiments: experiment A, 43.5 g/L; experiment B, 54.0 g/L; experiment C, 46.2 g/L; experiment D, 44.9 g/L; experiment E, 45.0 g/L; experiment F, 45.0 g/L; experiment G, 47.2 g/L. After preparation, the brews were rapidly cooled in an ice bath and immediately used for quantitative analysis.

Quantification of Di- and Trihydroxybenzenes in Coffee Brews. Following the methodology reported recently (*20*), an aliquot of a cooled coffee brew (20 mL) was placed into a centrifugation tube (80 mL) with a screw cap (Schott AG, Mainz, Germany), defined amounts of the labeled internal standards **d$_3$-1**, **d$_4$-3**, **d$_3$-4**, **d$_3$-5**, and **d$_3$-6** dissolved in methanol, were added, and, after the tube had been closed, the sample was equilibrated for 5 min at room temperature with stirring. The equilibrated sample was extracted with diethyl ether (20 mL) and centrifuged (3000 rpm, 10 min), and the organic layer was freed from solvent in vacuum (20 mbar). The residue obtained was taken up in methanol/water (1:1, v/v; 1 mL), membrane filtered (0.45 μm), and, finally, analyzed by means of HPLC-MS/MS.

Calibration. Solutions of the deuterated internal standards **d$_3$-1**, **d$_4$-3**, **d$_3$-4**, **d$_3$-5**, and **d$_3$-6** and the analytes **1**−**5** were prepared in five concentration ratios from 0.2 to 5.0 and used for HPLC-MS/MS analysis. Calibration curves were prepared by plotting peak area ratios of analyte to internal standard against concentration ratios of each analyte to the internal standard using linear regression. Hydroxyhydroquinone (**2**) was calibrated with **d$_3$-1** as the internal standard over a range of concentration ratios from 0.2 to 5.0.

High-Performance Liquid Chromatography−**Mass Spectrometry (HPLC-MS/MS).** The Agilent 1100 series HPLC system consisted of a pump, a degasser, and an autosampler (Agilent, Waldbronn, Germany) 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. The nebulizer gas was zero-grade air (45 psi), whereas the curtain gas was nitrogen (35 psi). The quadrupoles operated at unit mass resolution. For instrumentation control and data collection Sciex Analyst software (v 1.4) was used. Detection was performed in multiple-reaction monitoring (MRM) mode, recording the transition from the negative pseudo-molecular ion $[M - H]^-$ to the fragment after collision-induced dissociation. The following mass transitions of the fully labeled internal standards and the nonlabeled analytes were monitored: **1**, 5.52 min (*m*/*z* 125 → 69); d_3-**1**, 5.36 min (*m*/*z* 128 → 72); **2**, 3.77 min (*m*/*z* 125 → 79); **3**, 19.79 min (*m*/*z* 109 → 91); d_4-**3**, 19.19 min (*m*/*z* 113 → 94); **4**, 21.32 min (*m*/*z* 137 → 122); d_3-**4**, 21.29 min (*m*/*z* 140 → 125); **5**, 19.49 min (*m*/*z* 123 → 108); d_3-**5**, 19.41 min (*m*/*z* 126 → 111); **6**, 20.08 min (*m*/*z* 123 → 108); d_3-**6**, 19.97 min (*m*/*z* 126 → 111). After sample injection (5 μL), chromatographic separation was carried out on a 150 × 2.5 mm i.d. Luna Phenyl-Hexyl column (Phenomenex, Aschaffenburg, Germany) with gradient elution at a flow rate of 0.25 mL/min. Solvent A was 0.5% formic acid in methanol, and solvent B was 0.5% formic acid in water. For chromatography, solvent A was kept isocratically at 2% for 3 min and then increased linearly to 15% within 10 min, followed by a linear increase to 100% within additional 10 min, and, finally, maintained isocratically at 100% for 9 min.

RESULTS AND DISCUSSION

To gain first insights into the precursors generating di- and trihydroxybenzenes during coffee roasting, the intact raw bean was separated into the aqueous bean extractables (BE), yielding about 19.4 g per 100 g of coffee beans, and the fully extracted bean shell (BS), accounting for 80.6 g per 100 g of coffee beans, by using an exhaustive aqueous extraction procedure similar to those reported recently (*7*, *22*). This technology allowed an extraction of the water solubles without any prior grinding of the beans and opened the possibility of using the nonsoluble, intact bean shell as the "matrix" in model roasting experiments using a batch roaster. This gave us the possibility to infuse the freeze-dried bean shell with aqueous solutions of either single coffee compounds or fractions isolated from the raw bean solubles, to roast these reconstituted beans after freeze-drying, and finally to measure the activity of these compounds "in bean" as precursors of di- and trihydroxybenzenes.

In a first experiment (experiment A), a fully reconstituted coffee bean was prepared by re-incorporation of the raw BE in their "natural" concentration into the bean matrix (BS). These reconstituted beans (experiment A) were then roasted for 4.5 min at 240 °C and ground, and an aliquot of the freshly prepared brew was used for quantitative analysis of the di- and trihydroxybenzenes **1**−**6** by means of stable isotope dilution analysis (SIDA) as shown in **Figure 2**. As control, the amounts of the phenols were determined in a coffee brew prepared from bean shells (BS) only without any further additive. The results, given in **Table 2**, show that the fully reconstituted coffee brew (experiment A) contained all of the di- and trihydroxybenzenes

Table 2. Concentrations of Pyrogallol (**1**), Hydroxyhydroquinone (**2**), Catechol (**3**), 4-Ethylcatechol (**4**), 4-Methylcatechol (**5**), and 3-Methylcatechol (**6**) in Aqueous Brews Prepared from Roasted Precursor-Loaded Bean Shells

		concn[a] (mg/kg) of compound					
expt	precursor added[b]	**1**	**2**	**3**	**4**	**5**	**6**
control	no additive	0.58	nd[c]	0.35	0.07	0.01	0.03
A	bean extract	1.47 (± 0.12)	3.10 (± 0.21)	1.43 (± 0.10)	0.12 (± 0.02)	0.09 (± 0.01)	0.33 (± 0.02)
B	tCA extract[d]	1.14	3.18	0.82	0.13	0.03	0.06
C	5-*O*-caffeoylquinic acid	0.99	1.87	0.84	0.11	0.02	0.04
D	caffeic acid	0.58	nd	3.96	3.93	0.03	0.04
E	ferulic acid	0.11	nd	1.72	0.24	0.10	0.04
F	quinic acid	0.81	1.02	0.49	0.05	0.02	0.04
G	carbohydrates/amino acids	0.70	2.60	0.35	0.06	0.03	0.14

[a] The data given are the mean of five replicates. [b] Bean shells were loaded with the individual precursor components prior to roasting. [c] Not detectable. [d] Total chlorogenic acid (tCA) extract isolated from raw coffee beans.

but in rather different concentrations. By far the highest amounts of 3.1 mg/L were found for hydroxyhydroquinone (**2**), followed by pyrogallol (**1**) and catechol (**3**), for which 1.47 and 1.43 mg/L have been determined. Comparatively low amounts of 0.33 mg/L were observed for 3-methylcatechol (**6**), whereas 4-methylcatechol (**5**) and 4-ethylcatechol (**4**) were detected in concentrations of 0.09 and 0.12 mg/L only. Compared to experiment A, the coffee brew made from the roasted bean shells (control) contained significantly lower amounts of pyrogallol and catechol, whereas the alkyl-substituted catechols **4**−**6** were present just in trace amounts (**Table 2**). As these bean shells were entirely free of water soluble low molecular weight compounds, the high molecular weight bean matrix was also shown to exhibit some precursor potential for catechol and pyrogallol generation. These findings pinpoint matrix-bound phenolic acids, which have been recently suggested on the basis of alkaline hydrolytic experiments (*23*). However, comparison of the amounts of di- and trihydroxybenzenes present in the brew made of bean shells (control) with those present in experiment A revealed the raw bean water solubles as the more efficient precursors in di- and trihydroxybenzene generation during coffee roasting.

To gain further insights into the precursor potential of individual components of the raw bean water solubles, the tCA extract (experiment B) isolated from raw coffee beans, 5-*O*-caffeoylquinic acid (experiment C), caffeic acid (experiment D), ferulic acid (experiment E), and quinic acid (experiment F) were infused into the raw coffee bean shells (BS) as detailed in **Table 1**. After roasting and grinding of these precursor-loaded beans, aqueous brews were prepared by hot water percolation of the powder and immediately analyzed for their amounts of compounds **1**−**6** (**Table 2**).

Analysis of the brew made of the beans of experiment B (**Table 2**) revealed a phenol pattern for compounds **1**−**4** which was comparable with that found for experiment A and demonstrated the chlorogenic acids as the main precursors for pyrogallol (**1**), hydroxyhydroquinone (**2**), catechol (**3**), and 4-ethylcatechol (**4**). However, the tCA-loaded beans generated only trace amounts of compounds **5** and **6**, thus indicating besides chlorogenic acids the existence of another precursor for 3-methylcatechol and 4-methylcatechol in the raw bean extract (fraction BE). Except for hydroxyhydroquinone (**2**), the brew prepared from roasted 5-*O*-caffeoylquinic acid loaded beans (experiment C) generated a phenol pattern comparable with that found for experiment B (**Table 2**). The higher amount of hydroxyhydroquinone formed in experiments A and B indicates either the existence of another phenolic precursor besides the caffeoyl quinic acids or a synergistic interplay of the different mono- and dicaffeoylquinic acids present in the tCA extract (experiment B).

To investigate the precursor potential of the caffeic acid moiety in 5-*O*-caffeoylquinic acid, the bean shells were loaded with corresponding amounts of caffeic acid (experiment D) prior to roasting. Quantitative analysis of the di- and trihydroxyphenols in the brew prepared from experiment D revealed rather high amounts of catechol (**3**) and 4-ethylcatechol (**4**), the amounts of which exceed those by a factor of 4 or 36 those found for experiment C (**Table 2**). It is interesting to notice that, compared to the control experiment, 3-methylcatechol and 4-methylcatechol were formed in just trace amounts and not even trace amounts of hydroxyhydroquinone and pyrogallol were generated from caffeic acid (**Table 2**). Although the data obtained from experiment D clearly demonstrate that "free" caffeic acid is a more efficient precursor for catechol and 4-ethylcatechol than the caffeic acid moiety bound in 5-*O*-caffeoylquinic acid, a comparison with the phenol pattern of the coffee beans of experiments A and B, in which only low amounts of 4-ethylcatechol could be found, are contradictory to the major involvement of "free" caffeic acid. Therefore, these findings did not support the chlorogenic acid degradation route proposed by Leloup et al. (*19*) assuming the hydrolysis of mono- and di-*O*-caffeoylquinic acids prior to further thermal degradation of the liberated caffeic acid to give catechol and 4-ethylcatechol (**Figure 1**).

Loading the bean shell with ferulic acid (experiment E) prior to roasting revealed a similar picture as in experiment D, the target phenols being formed only in lower concentrations, thus demonstrating the higher precursor potential of bound caffeic acid and caffeoylquinic acids in thermal di- and trihydroxyphenol generation.

Finally, the bean shells were infused with quinic acid (experiment F) prior to roasting. Quantitative analysis of the phenols in the aqueous brew revealed quinic acid as a precursor of hydroxyhydroquinone (**2**) as the main phenol, followed by pyrogallol (**1**) and catechol (**3**). Compared to the control, the formation of hydroxyhydroquinone was exclusively observed in experiments A−C and F and was favored when the tCA extract or 5-*O*-caffeoylquinic acid was used as the precursor, thus demonstrating that this trihydroxybenzene is also mainly originating from the quinic acid moiety of chlorogenic acids in coffee.

Taking all of these data into account, it can be concluded that the caffeic acid moiety of chlorogenic acids is generating catechol (**3**), 4-ethylcatechol (**4**), and 4-methylcatechol (**5**) upon bean roasting, whereas the quinic acid moiety is converted into pyrogallol (**1**), hydroxyhydroquinone (**2**), and catechol (**3**). In contrast to the phenols **1**−**5**, not a single phenolic precursor could be identified to generate higher amounts of 3-methylcatechol (**6**).

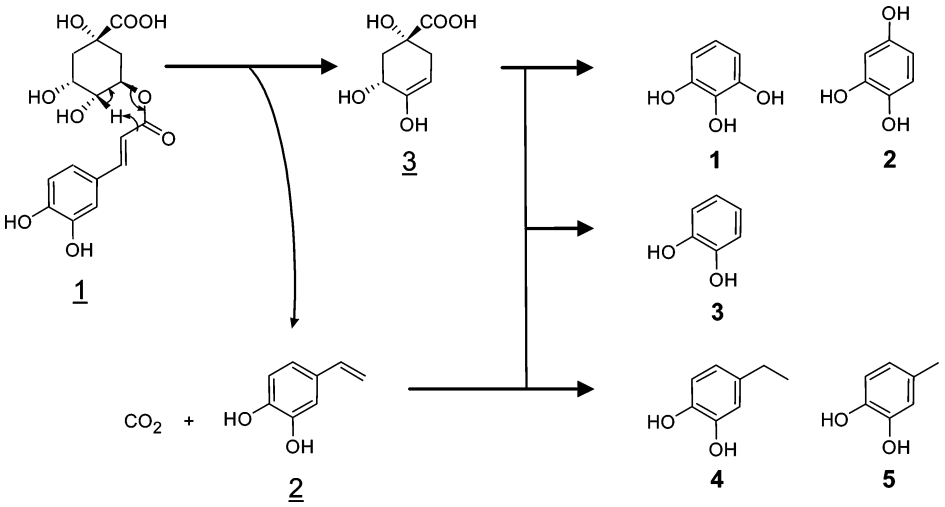

Figure 3. Reaction scheme proposed for the formation of pyrogallol (**1**), hydroxyhydroquinone (**2**), catechol (**3**), 4-ethylcatechol (**4**), and 4-methylcatechol (**5**) via thermal *syn*-elimination of 5-*O*-caffeoylquinic acid (1).

To investigate whether 3-methylcatechol is generated upon coffee roasting via Maillard-type reactions, an aqueous solution of the four quantitatively predominating carbohydrates and six amino acids, each in natural concentration (**Table 1**), was infused into the bean shell prior to roasting (experiment G). Quantitative analysis of the phenols **1**−**6** in the brew prepared from experiment G revealed the amino acid/carbohydrate mixture as the only precursor system that was found to generate noticeable amounts of 3-methylcatechol (**6**). Whereas 0.14 mg/L of 3-methylcatechol was detected in experiment F, the amounts of this phenol liberated from tCA extract, 5-*O*-caffeoylquinic acid, caffeic acid, ferulic acid, or quinic acid were below 0.06 mg/L. Even more interesting, the carbohydrate/amino acid mixture generated huge amounts of hydroxyhydroquinone (**2**); for example, with an amount of 2.6 mg/L the Maillard reaction was more efficient in the production of compound **2** than the 5-*O*-caffeoylquinic acid, generating just 1.87 mg/L. In addition, pyrogallol (**1**) was generated by the "in bean" model roasting of carbohydrates and amino acid, thus confirming previous findings (*14*).

It can be concluded that upon coffee bean roasting catechol (**3**) is primarily formed by degradation of caffeoylquinic acids from both parts of the molecule, the caffeic acid and the quinic acid moiety, as well as from Maillard-type reactions from carbohydrates and amino acids. In contrast, pyrogallol (**1**) and hydroxyquinone (**2**) are efficiently generated from carbohydrates and amino acids and, in addition, from free or chlorogenic acid bound quinic acid moieties. 4-Ethylcatechol (**4**) is exclusively generated upon thermal breakdown of caffeic acid moieties. 3-Methylcatechol (**6**) is formed primarily from the Maillard reactions and, to a minor extent, also from various phenolic precursors, whereas 4-methylcatechol (**5**) is produced in only trace amounts from all of the different precursors investigated.

From these results, it can be concluded that 5-*O*-caffeoylquinic acid (1) via a thermal *syn*-elimination is degraded to give the phenols **1**−**5** as outlined in **Figure 3**. Via the putative breakdown product 4-vinylcatechol (2), the degradation of the caffeic acid moiety gives rise to catechol (**3**), 4-ethylcatechol (**4**), and 4-methylcatechol (**5**), whereas pyrogallol (**1**), hydroxyhydroquinone (**2**), and also catechol (**3**) originate from the quinic acid moiety, most likely via intermediate 3.

Stable isotope labeling experiments are currently in progress to elucidate the reaction pathways channeling the degradation of carbohydrates into the phenols **1**−**3** and to gain first insights into the mechanisms of 3-methylcatechol formation.

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