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Interactions between Volatile and Nonvolatile Coffee Components. 2. Mechanistic Study Focused on Volatile Thiols

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This study is the second of two publications that investigate the interactions between volatile and nonvolatile components in coffee brew. The purpose here was to shed some light into the chemical mechanisms responsible for the decrease of volatile thiols when in contact with coffee nonvolatiles. A mixture of volatile thiols covering a large range of physicochemical properties was monitored over time in the presence of a coffee brew model. The binding potential was estimated by SPME-GC-MS. Additives inhibiting specific reaction pathways were preincubated with the coffee brew 1 h prior to addition of the volatile compounds. Degradation kinetics of the volatile thiols were characterized by their rate constants *k*(obs). The effect of individual additives was shown by calculating *k*(rel), the relative rate constant as compared to the reference without additive. The conclusion was that thiols, mainly responsible for the "roasty" and "burnt" notes, disappear via two main chemical mechanisms. The results suggest that nucleophilic addition is the major pathway for thiol degradation. Addition occurs on oxidized species generated in the matrix in the presence of air. This mechanism prevails for aliphatic thiols (e.g., ethanethiol, methanethiol). Benzylic thiols (such as 2-furfurylthiol) can react in parallel via another pathway that is slowed in the absence of oxygen and in the presence of a radical scavenger. This points to a radical mechanism, but further work is needed to support this hypothesis. A direct correlation between thiol hydrophobicity and the magnitude of the interactions was shown as well. Therefore, weak physical interactions or hydrophobic assistance accelerating chemical reactions cannot be excluded at this point of the study.

KEYWORDS: Headspace analysis; SPME; coffee; aroma; thiols; mechanism; radical reactions; nucleophilic addition; oxidation

INTRODUCTION

Coffee aroma is the result of a complex balance of about 800 volatile compounds mainly formed during the roasting process. During the past decade, a subset of aroma impact compounds were identified (*1*) and reconstituted in model systems simulating real coffee aroma to a large extent (*2*). Further studies have shown that coffee aroma rapidly loses its fresh, sulfury notes after roasting (*3*) and especially after the preparation of the coffee brew (*4*−*6*). Among the coffee aroma components, thiols (methanethiol, 2-furfurylthiol, 3-methyl-2-butenethiol, 3-mercapto-3-methylbutyl formate) are both the most affected in the presence of the coffee matrix and also among the most important in terms of sensory perception (*2*). Thiol losses may be explained by physical or chemical interactions with coffee nonvolatile components. Physical interactions include hydrophobic trapping, salting out, and chemical interactions reversible or irreversible covalent binding. In a previous paper (*7*), we showed that (1) melanoidins are the main responsible components for thiols degradation in coffee beverages, (2) chlorogenic acids and protein-like materials play a minor role in the overall interactions, and (3) the other nonvolatile components are inert toward thiols. Based on these results and reported chemical reactivities of thiols in model or food systems, **Figure 1** summarizes possible reaction pathways for thiol losses in coffee. They can be detailed as follows.

Radical reactions can be initiated by oxidation, transition metal catalysis, light, or heat treatment. In the presence of oxygen and water, coffee matrix generates reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radicals ($O_2^{\bullet -}$), and hydroxyl radicals ($HO^{\bullet}$) (*8*). H_2O_2 can act as a source of hydroxyl radicals via metal-catalyzed Fenton reactions (*9*). The hydroxyl radical is the most reactive species and easily abstracts a hydrogen radical from nonvolatile or volatile coffee components. The energy needed for the hydrogen abstraction from various sensitive aroma components was predicted by ab initio calculations (*10*). The superoxide radical

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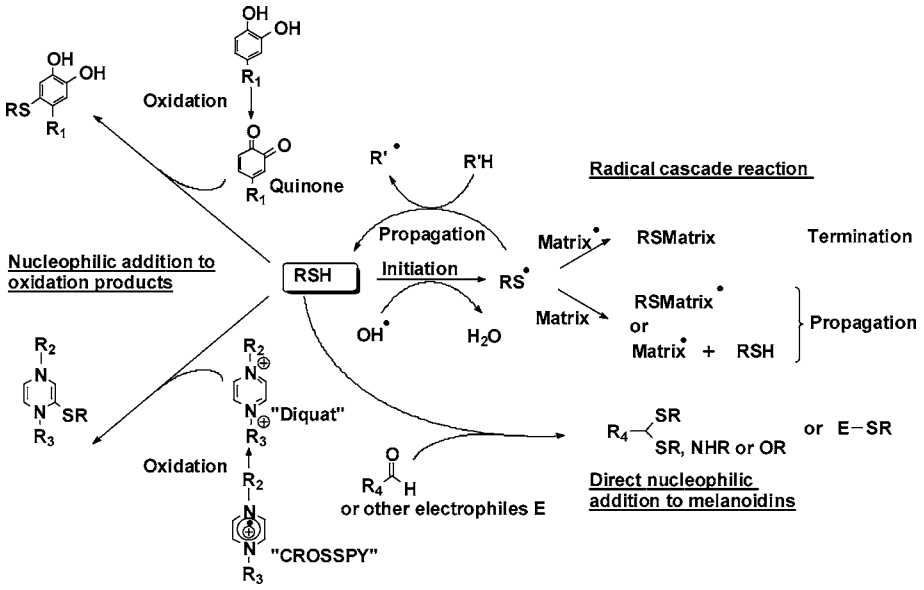

Figure 1. Possible mechanistic pathways for the degradation of thiols exposed to nonvolatile coffee components in solution.

is a less reactive species but is still able to break some low-energy C−H or S−H bonds. RS• (thiyl radicals) may add to electron-rich double bonds or abstract a hydrogen atom to form any weaker radical in terms of formation energy, thereby starting a cascade (propagation). Radicals may also recombine with other radicals from volatile compounds or more likely from the coffee matrix (termination). The degradation of 2-furfurylthiol (FFT) in Fenton-type model systems has recently been studied (*11*). Its degradation rate was also found to be positively correlated with the radical activity of the coffee solution as determined by ESR (*4*).

Ionic reactions involve a two-electron transfer between an electron donor (nucleophile) and an electron acceptor (electrophile). Thiols are nucleophiles, and the coffee matrix presents many electrophilic addition sites. Some of these electrophiles are formed by oxidation. For example, (poly)phenols generate semi-quinones and quinones under oxidative conditions (*12*). Quinones readily undergo [1,4]-addition by nucleophilic volatiles such as thiols (*13*, *14*). In model systems, Hofmann et al. provided evidence that FFT can covalently bind to pyrazinium dications, the oxidation products of 1,4-bis-(5-amino-5-carboxy-1-pentyl)pyrazinium radical cations or "CROSSPY" (*4*). Because the CROSSPY species was also postulated in coffee as a key intermediate in roasting-induced melanoidin genesis (*15*), it may contribute to thiol degradation. Thiols might also add to other electrophiles found among coffee melanoidins (carbonyl compounds or other electron-poor heterocycles). These molecules do not depend on the presence of oxygen for their formation.

The goal of this work was to determine the mechanisms controlling the degradation of volatile thiols in coffee, especially the contribution of the individual chemical reaction pathways to the overall interaction, the identification of possible synergies between the mechanistic pathways, and the contribution of physical and chemical interactions to the overall interactions measured. The experimental approaches to answer the above questions were (1) the screening of a variety of volatile thiols with different physicochemical properties and (2) the use of additives inhibiting or favoring specific reaction pathways (nucleophilic competitor, reducing agents, radical scavengers, and Fenton inhibitor) in the presence or absence of oxygen.

Table 1. Preparation of Coffee Brews with Additives[a]

additives	expected action	additive stock solutions [mg/mL]	additive final concentrations [mmol/g dry coffee]
hydroxylamine × HCl	nucleophilic competitor	25, 50, 100, 150	3.6, 7.2, 14.4, 21.6
ascorbic acid (Na salt)	radical scavenger	2, 5, 10, 20, 50	0.1, 0.5, 1, 2, 5
caffeic acid	oxygen scavenger	1, 2, 5	0.05, 0.11, 0.28
DTPA	metal chelator	3.9–19.6	0.1, 0.5
$Na_2S_2O_3 \cdot 5H_2O$	reducing agent	2.5, 25, 125	0.1, 1, 5
Na_2SO_3	nucleophile and reducing agent	1.3, 6.3, 12.6	0.1, 0.5, 1

[a] Concentrations of stock solutions and final concentrations in sample for interaction measurement.

MATERIALS AND METHODS

Coffee Sample. Coffee brew models were produced from a blend of Arabica 80% and Robusta 20% as described earlier (*7*). The medium roasted extract (CTN 85) with extraction yield 22.7% was used for this study. Coffee stock solutions were prepared at 2.5% total solid (t.s.) in acetate buffer 0.01 M at pH 5.2.

Additives. All chemicals were purchased from Fluka/Aldrich/Sigma (Buchs, Switzerland) and used without further purification. Stock solutions of the additives (hydroxylamine hydrochloride, l-ascorbic acid sodium salt, caffeic acid, sodium thiosulfate pentahydrate, sodium sulfite anhydrous, diethylenetriaminepentaacetic acid DTPA) were prepared in the working buffer (0.01 M acetic acid, pH 5.2) as described in **Table 1**. When needed, the stock solutions were adjusted to pH 5.2 with HCl or NaOH.

Volatile Compounds. Volatile thiols were chosen to give mechanistic information on the interactions between thiols and coffee matrix components. The thiol stock solution was prepared in a glovebox (Easy Box EB 80-1 spez., MecaPlex, Switzerland, nitrogen atmosphere) in the working buffer at twice the final concentrations listed below. This stock solution was further diluted (1:1) either with the working buffer (blank samples) or with coffee brew, or with coffee brew + additive solution. The final concentrations of volatile thiols at t_0 were [μmol/L]: 2-methyl-2-propanethiol (2M2P), 3.7; 3-mercapto-3-methylbutyl formate (MMBF), 4.9; 2-butanethiol (2BT), 3.7; ethanethiol (EtSH), 5.4; propanethiol (PropSH), 4.4; butanethiol (BuSH), 3.7; pentanethiol (PentSH), 3.2; 2-furfurylthiol (FFT), 3.9; benzylthiol (BnSH), 3.4; thiophenol (PhSH), 9.7. They were chosen to be in the linear range of the SPME fiber and at a ppm level. At room temperature (RT), the

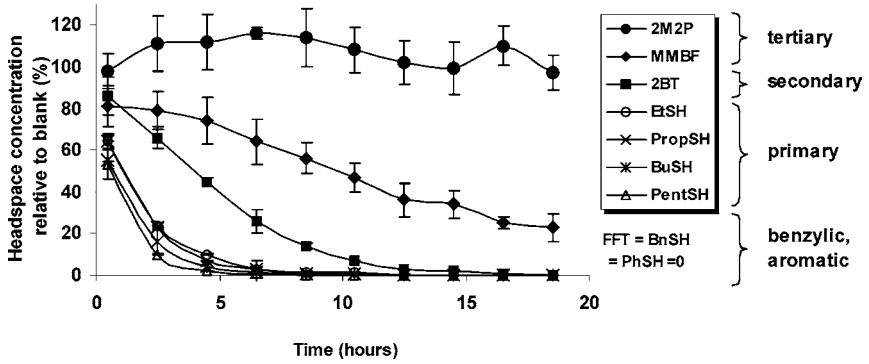

Figure 2. Degradation kinetics of various thiols in the presence of reconstituted coffee brew (coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2).

mixture containing the nine thiols in the working buffer with air as headspace was shown to be stable over 24 h.

Measurement of the Interactions. Interactions between thiols and coffee brews without additives were measured as described earlier (*7*). *Blank sample with additive (no coffee added) **A***: Thiols stock solution, additive stock solution, and working buffer were mixed in a ratio 5:1:4. The mixture was stirred 15 min at RT. 800 μL was transferred into a 2 mL amber silane-treated glass vial and equilibrated 1 h on the autosampler before headspace analysis. *Coffee solution with additive **B***: The coffee stock solution (2.5% t.s.) was diluted 4:1 with the additive stock solution. The mixture was stirred for 1 h at 25 °C and diluted 1:1 with the aroma mixture. After 15 min of stirring, an aliquot (800 μL) was filled into a 2 mL vial and equilibrated 1 h at 25 °C before headspace analysis. For kinetic studies, vials containing blank samples **A** and coffee samples **B** were prepared at time zero and put alternatively on the autosampler so that the headspace was sampled every 2−4 h in intact "aged" vials. Headspace concentrations of vials **A** and **B** were measured after the same time. The ratio of the two integration surfaces was plotted as a function of additive concentration and/or time for each additive. Na_2SO_3 and $Na_2S_2O_3$ both decreased the headspace concentration of the blank thiol mixture even upon short time equilibration (1 h). The results with these additives are therefore expressed relative to a blank thiol mixture solution without additive. For all of the other trials with additives, the results were expressed relative to a blank + additive to keep all parameters constant except for the coffee matrix. Trials under anaerobic conditions were entirely prepared in a glovebox (Easy Box EB 80-1 spez., MecaPlex, Switzerland) and with previously degassed buffer (Ar bubbling for 1 h). The headspace analysis of the samples was conducted by SPME-GC-MS as described earlier (*7*). Briefly, the instrumentation included a Varian CP-820 autosampler, a Hewlett-Packard 5973 gas spectrometer with a DB-Wax column (J&W Scientific, 30 m, 0.25 mm i.d., 0.25 μm film, 0.9 mL/min constant flow). The headspace of the vials was sampled with a PDMS/DVB fiber (65 μm, Supelco, Buchs, Switzerland) during 1 min equilibration. Aroma compounds were desorbed for 5 min at 240 °C, in the unsplit mode for the last 2 min. The GC gradient was 35 °C (3 min), 35−170 °C at 4 °C/min, 170−220 °C at 20 °C/min, and 220 °C for 10 min. Mass spectra were acquired in scan mode from 29 to 300 amu.

Kinetic Data Treatment. The data were treated assuming pseudo first-order kinetics. For each volatile compound, the ln of concentration was expressed as a function of time [s]. The slope of the curve gave $-k$(obs), the observed rate constant. The individual k(obs) for each volatile compound was then expressed as k(rel) relative to the corresponding k(ref), the rate constant in the coffee sample without additive: $k(\text{rel}) = k(\text{obs})/k(\text{ref})$.

Statistical Analysis. Each data point was measured in duplicate, and the error bars are deviations from average.

RESULTS

In all of the figures shown in this section, 100% refers to a blank sample treated exactly like the corresponding coffee sample (thiol mixture + buffer + additive or specific atmosphere and same reaction time) but without coffee added. The sample named reference is the coffee at pH 5.2 with the volatile thiol mixture under air and without additive. The data points were obtained under air unless specified otherwise.

Thiols in the Presence of Coffee Brew. A set of thiols with different hydrophobicities, steric hindrance of the thiol group, and chemical reactivities was selected. The behavior of these compounds in the presence of the reconstituted coffee brew was monitored over time (**Figure 2**). The thiols can be divided into four groups with increasing reactivity: tertiary thiols < secondary thiols < primary thiols < aromatic or benzylic thiols. Among the primary aliphatic thiols, the interactions increase with increasing hydrophobicity (from EtSH to PentSH). Within the group of the tertiary thiols, the bifunctional 3-mercapto-3-methylbutyl formate (MMBF) shows clearly higher interactions than the monofunctional 2-methyl-2-propanethiol (2M2P). Aromatic and benzylic thiols were already undetectable at the first datapoint.

Competitor Nucleophile. Hydroxylamine is one of the strongest nucleophiles known and will react with electrophilic counterparts including carbonyl compounds. When hydroxylamine was added to the coffee solution 1 h prior to the thiol mixture, the magnitude of the interactions with the thiols was decreased (**Figure 3**). This effect was concentration dependent, and for all primary, secondary, and tertiary thiols it leveled off above 8−10 mmol hydroxylamine/g coffee solids. 2-Furfurylthiol, a benzylic thiol, is further stabilized even above this concentration of hydroxylamine. This experiment is an indirect method to measure the number of electrophilic sites of the matrix (in this case 8−10 mmol/g dry coffee). These electrophilic sites include also reducing sugars, which present moderate to low reactivity toward thiols. The reaction of hydroxylamine with the coffee matrix is almost completed after only 1 h. Indeed, when the preincubation of the coffee solution with hydroxylamine was extended from 1 to 24 h, the stabilization of the sulfur volatiles was not significantly increased (**Figure 4**). Only FFT was clearly stabilized by the additional 23 h of reaction between hydroxylamine and matrix.

Role of Oxidation/Radical Reactivity. *Inert Atmosphere and Reducing Agents.* As compared to aerobic conditions (**Figure 2**), thiol degradation was drastically slowed under nitrogen in the presence of reconstituted coffee brew (**Figure 5**). Therefore, oxidation was suspected to play a major role in thiol degradation. Two reducing agents were investigated, sodium sulfite and sodium thiosulfate (**Figure 6**). These experiments were conducted under air. Sodium sulfite is a potent oxygen scavenger under neutral and basic conditions. It is broadly applied for shelf life extension in food industry (*16*). Sodium thiosulfate is known to quench peroxides and hydroperoxides. The stability of volatile thiols in the presence of coffee brew was compared with and without the two reducing agents. The effect of the two reducing

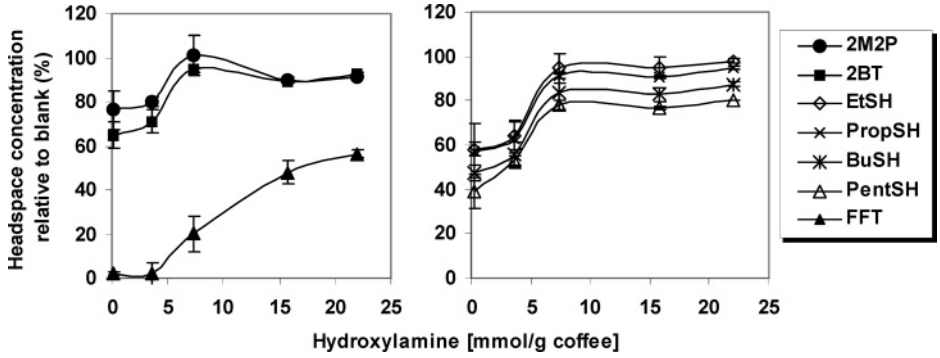

Figure 3. Inhibition of thiol interactions by addition of competitor nucleophile hydroxylamine. Preincubation with hydroxylamine 1 h, equilibration with volatile thiols 1 h, coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2.

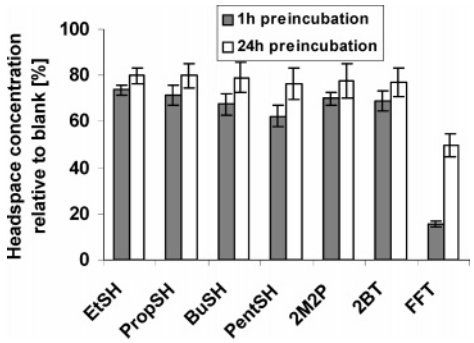

Figure 4. Inhibition of thiol–matrix interactions by pretreatment with nucleophile hydroxylamine. Effect of preincubation time (1 h, 24 h), equilibration with volatiles 1 h, coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2.

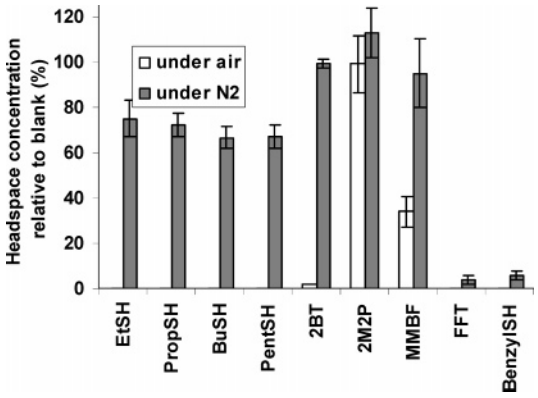

Figure 5. Comparison of thiol levels after 15 h under air and under N_2 (coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2).

agents was radically different. Sodium sulfite proved to be an efficient stabilizing agent, while the magnitude of the interactions was stronger in the presence of sodium thiosulfate than in the reference coffee brew without additive.

Radical Scavenger and Fenton Inhibitor. Low molecular weight coffee fractions show the highest antioxidant and radical scavenging potential (*17*, *18*). They were also identified as the most reactive toward sensitive aroma components (*4*). If thiol degradation occurs via oxidation and/or radical reactions, coffee intrinsic radical or oxygen scavengers do not prevent this degradation from taking place. These findings led us to select a series of additives to favor/inhibit specific oxygen/radical related reaction pathways. Ascorbic acid acts as a potent water-soluble antioxidant by scavenging free radicals such as hydroxyl, peroxyl, and hydroperoxyl radicals (*19*). It can also regenerate other small antioxidant molecules from their radical species, for example, thiyl radicals. Depending on the conditions, it might be pro-oxidant when it reduces transition metals regenerating active species for the Fenton reaction. The presence of >1 mmol ascorbic acid/g dry coffee, 1 h prior to the addition of the thiol mixture, strongly decreased the magnitude of the interactions (**Figure 7**). However, this stabilizing effect was less important than the one observed in the absence of oxygen (**Figure 5**). The effect of ascorbic acid was concentration dependent and leveled off above 0.5 mmol/g dry coffee. For FFT, the stabilization was only moderate and did not totally level off in the concentration range tested. Caffeic acid is an oxygen quencher but might also participate in the generation of ROS when undergoing oxidation in the presence of air (*20*). Its effect on the stability of volatile thiols was moderate to nonsignificant (**Figure 8**). Due to its low water solubility, it was only tested in a narrow concentration range. DTPA is a transition metal chelator often applied to inhibit Fenton reaction by Fe(II) complexation. It therefore slows down or inhibits the formation of hydroxyl radicals from hydrogen peroxide (*21*). In our model system, it showed a weak concentration-dependent stabilizing effect on the thiol mixture (**Figure 8**).

Inert Atmosphere and Competitor Nucleophile Combined. The model thiol mixture was strongly stabilized in the absence of O_2 (**Figure 5**). This stabilization was further increased when a competitor nucleophile was added to the coffee solution 1 h prior to the thiols and the whole procedure was carried out under inert atmosphere (**Figure 9**).

DISCUSSION

Role of Nucleophilic Addition. The rate constants of thiol decay increase with increasing nucleophilicity of the thiol function: tertiary < secondary < primary (**Table 2**; **Figure 2**). This reactivity ranking is mainly due to decreasing steric hindrance from the tertiary to the primary thiol. The trend only applies to aliphatic thiols. Indeed, 2-furfurylthiol and benzylthiol present faster kinetics than all of the other primary aliphatic thiols (e.g., ethanethiol). Indeed, both of them were undetectable after only 1 h exposure to the coffee matrix. This suggested that these "benzylic" thiols undergo another mechanistic pathway predominantly or in parallel with the nucleophilic degradation. The tertiary thiol MMBF (3-mercapto-3-methylbutyl formate) should be considered separately. Indeed, it is a bifunctional compound comprising an ester and a thiol. Its higher reactivity as compared to the simple tertiary thiol (2M2P) is due to hydrolysis of its ester function as was shown previously (*22*).

Aromatic thiols (thiophenol) are more acidic than aliphatic thiols (EtSH), and electron-donating groups decrease the acidity. Dmuchovsky et al. showed that the most acidic thiol gives the

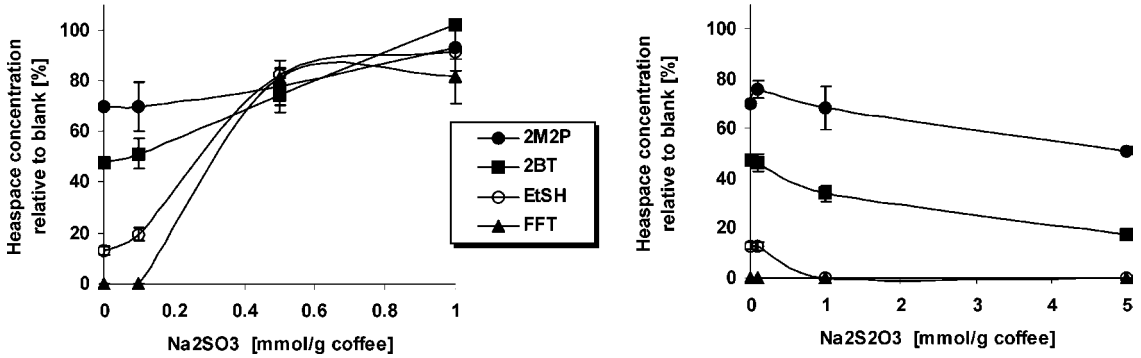

Figure 6. Effect of reducing agents Na_2SO_3 and $Na_2S_2O_3$ on the stability of thiols in the presence of reconstituted coffee brew (1% t.s. in acetate buffer 0.01 M, pH 5.2) measurement after 1 h.

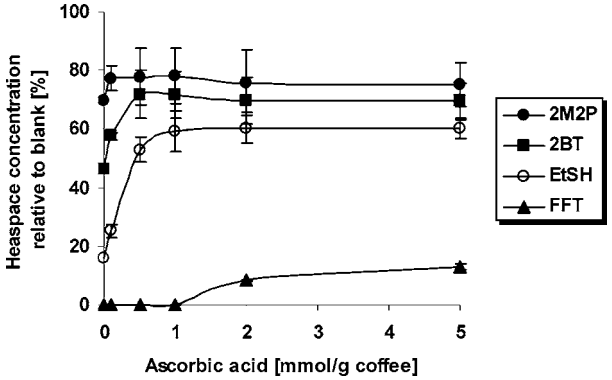

Figure 7. Inhibition of thiol/matrix interactions by addition of a radical quenching reagent: ascorbic acid. Preincubation with ascorbic acid 1 h, equilibration with volatile thiols 1 h, coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2.

Table 2. Rate Constants of Thiol Degradation in the Presence of Reconstituted Coffee Brew, t.s. 1%, pH 5.2; See Also **Figure 2**

flavor compounds	type	rate constant k_{ref} [mol^{-1} s^{-1}]	pK_a	relative nucleophilicity (*24*)
PhSH/PhS^-	aromatic	$>7.70 \times 10^{-04}$	8.6,[a] 6.5[b]	
FFT	primary/benzylic	$>7.70 \times 10^{-04}$	11.3[a]	1400
BenzSH	primary/benzylic	$>7.70 \times 10^{-04}$	11.8[a]	1200
PentSH	primary	1.83×10^{-04}		
BuSH	primary	1.42×10^{-04}	12.6[a]	1000
PropSH	primary	1.32×10^{-04}		
EtSH	primary	1.19×10^{-04}		
2BT	secondary	8.11×10^{-05}	12.9[a]	380
MMBF	tertiary	2.12×10^{-05}		
2M2P	tertiary	1.02×10^{-06}	13.1[a]	340

[a] In acetone/H_2O (3:1 v/v). [b] In water (*23*).

highest rate of addition to maleic anhydride, indicating that the dissociation equilibrium between thiol and thiolate dominates the overall rate of addition (*23*). Due to its low pK_a (6.5), thiophenol is the only thiol of our test group to be significantly deprotonated at coffee pH (~5). The corresponding thiophenolate is an excellent nucleophile, higher in reactivity than the aliphatic primary thiols in their protonated form. This explains why its degradation kinetics was among the fastest within the thiol group tested in this study.

The competitor nucleophile hydroxylamine blocks the electrophilic reactive sites in the coffee matrix and therefore prevents the thiols from reacting via this pathway (**Figure 3**). Two different behaviors were shown for the aliphatic and the benzylic thiols. The aliphatic thiols (e.g., ethanethiol) were dramatically stabilized when 8−10 mmol of hydroxylamine/g dry coffee was added 1 h prior to the contact between thiols and matrix. Only FFT did not follow the same pattern. This benzylic thiol still degraded even in the presence of 7−21 mmol of hydroxylamine/g dry coffee, suggesting that a parallel reaction mechanism occurs. At this point of the study, we concluded that the reaction between aliphatic thiols (e.g., ethanethiol) and the coffee matrix is mainly due to nucleophilic additions. The addition might occur on electrophiles present as such in the matrix or formed by oxidation (**Figure 1**).

Table 3. Rate Attenuation ($1/k_{Rel}$) of Thiol Losses Observed in the Presence of Various Additives

additive	amount (mmol/g)	EtSH	PropSH	BuSH	PentSH	FFT
aerobic		1.0	1.0	1.0	1.0	1.0
anaerobic		17.9	19.8	15.9	16.4	45.1
hydroxylamine	10	10.5	11.4	10.8	13.4	4.0
anaerobic + hydroxylamine[a]	21	64.8	82.5	101.3	236.1	92.6
anaerobic + hydroxylamine[b]	21	3.6	4.2	6.4	14.4	4.1
Na_2SO_3	1	51.5	61.7	18.4	62.7	67.9
ascorbic acid	1	9.0	7.8	4.4	3.7	23.6
caffeic acid	0.28	0.3	0.3	0.3	0.3	1.0
DTPA	0.5	0.5	0.7	0.7	1.0	1.0

[a] Relative to aerobic reference without hydroxylamine. [b] Relative to anaerobic without hydroxylamine.

Role of Oxidation/Radical Reactivity. In the absence of oxygen, linear aliphatic thiols are only 20−30% degraded after 15 h (**Figure 5**). This means a 15−20 times attenuation factor of the reaction rate as compared to the reference system under air. Under the same conditions, the degradation of FFT is slowed more than 40 times (**Table 3**). This stabilization suggests that either thiols add to oxidation products that are generated in the coffee matrix in the presence of oxygen, or oxygen is needed to create a reactive environment (e.g., radical species) involved in thiol degradation. In the presence of a weak reducing agent like sodium thiosulfate, the thiol mixture was unaffected or slightly destabilized depending on concentration. Therefore, the trapping of ROS species is not sufficient to prevent degradation of the thiols under investigation. Many other radical species are present in the beverage to maintain the radical cascade even under these conditions (*24*). In the coffee beverage (pH ≈ 5), sodium sulfite acts as reducing and oxygen scavenging reagent. In parallel, it reacts with matrix electrophiles (e.g., aldehydes). This mechanism was previously mentioned for the stabilization of other food systems such as beer (*25*, *26*). In beer, both free and bound sulfites contribute to the elimination of oxygen and reactive oxygen species present in the complex medium. Furthermore, sodium sulfite might also interfere with radical cascades. The observed stabilization is therefore due to a cumulative effect on all of these mechanistic pathways.

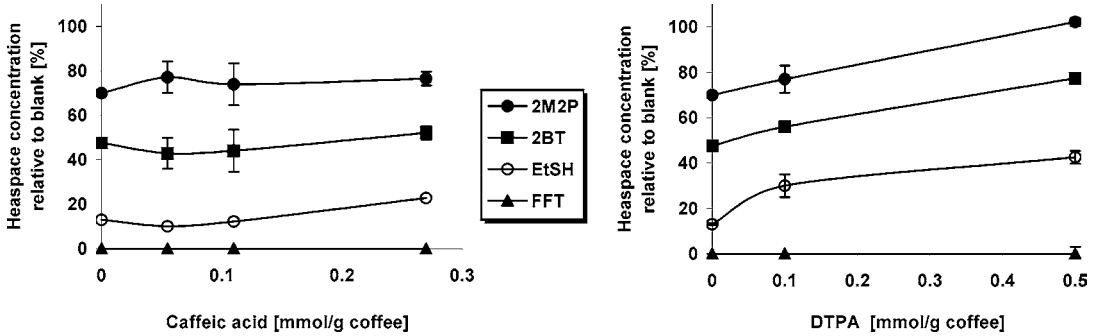

Figure 8. Effect of caffeic acid and DTPA on thiol/matrix interactions. Preincubation with additive, 1 h, equilibration with volatile thiols 1 h, coffee at 1% t.s. in acetate buffer 0.01 M, pH 5.2.

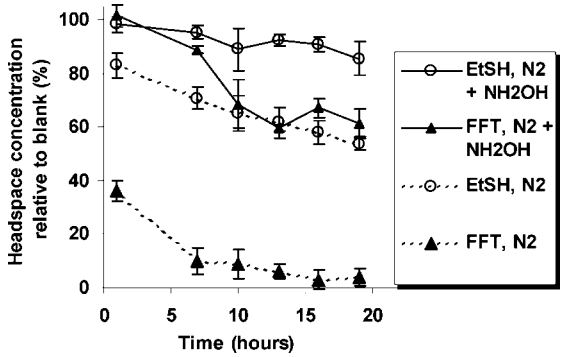

Figure 9. Separate and cumulative effect of competitor nucleophile (NH_2OH, 21 mmol/g coffee) and inert atmosphere (N_2) on the stability of thiols in the presence reconstituted coffee brew (1% t.s., acetate buffer 0.01 M, pH 5.2, measurement after 1 h).

For all of the aliphatic thiols, the stabilization was stronger in the absence of oxygen than in the presence of ascorbic acid (**Table 3**). This suggests that the radical environment does not play the major role in the degradation of aliphatic thiols (e.g., EtSH) and the nucleophilic addition to oxidation products in the matrix is more plausible. For these compounds, the addition of hydroxylamine resulted in a stabilization close to the one obtained in the absence of oxygen. Both parameters seem to be equally necessary to lead to the degradation. For the benzylic thiol FFT, the observed stabilization under inert atmosphere and in the presence of ascorbic acid is larger than that in the presence of the competitor nucleophile (**Table 3**). This suggests that the instability of FFT is not only due to addition to electrophilic sites but also to reactions involving oxidation and possibly radical reactions.

In summary, the degradation of aliphatic thiols (primary, secondary, tertiary) is mostly controlled by nucleophilic reactivity but needs also oxidizing conditions, while benzylic thiols (FFT, benzylthiol) also degrade by other reactions involving oxidation and inhibited by radical scavenger ascorbic acid.

Synergistic Effect between Different Reactions Pathways. When the interaction measurements were carried out under inert atmosphere and with the nucleophile additive, an addition of the two stabilization effects was observed (**Figure 9**). This suggests that both electrophilic addition sites and oxygen or a radical reaction medium are needed for the degradation of thiols. The first aspect is more important for aliphatic thiols, and both are important for the degradation of benzylic thiols such as FFT. This can be seen from the relative stabilizations obtained (1) under nitrogen, (2) with hydroxylamine, and (3) under nitrogen with hydroxylamine: for FFT $1/k_{rel} = 45$, 4, and 93; EtSH $1/k_{rel} = 18$, 11, and 65 (**Table 3**). These findings led us to the mechanistic conclusions described below.

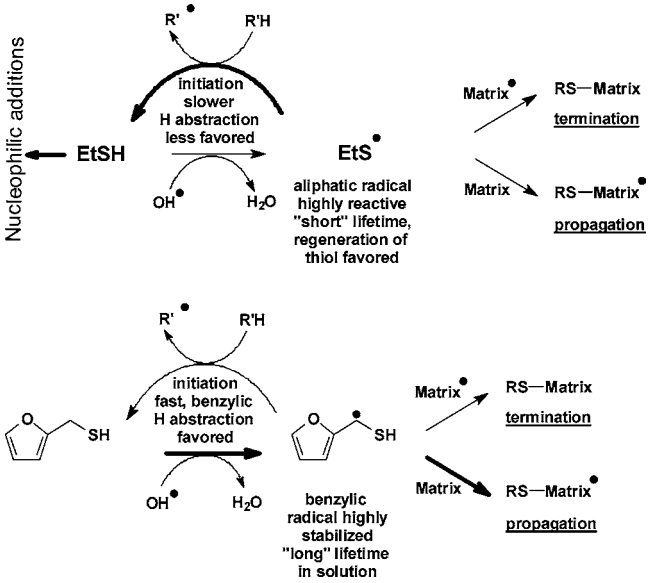

Figure 10. Proposed pathways for the degradation of aliphatic thiols (e.g., EtSH). Nucleophilic additions prevail, and benzylic thiols (e.g., 2-furfurylthiol)–radical mechanisms play a significant role. Favored steps are depicted in bold.

Proposed Mechanisms for Thiol Degradation in the Presence of Coffee Matrix. On the basis of the above findings, we propose that aliphatic thiols (primary, secondary, and tertiary) mainly degrade by nucleophilic addition to the coffee matrix. These compounds may generate the corresponding thiyl radicals under radical reaction conditions (e.g., upon $H^{\bullet}$ abstraction by $OH^{\bullet}$). However, these radicals are highly reactive and therefore the abstraction of an H atom from the matrix is kinetically favored and the thiols regenerated. Statistically, these species will be present most of the time in their neutral RSH form. This allows them to degrade mainly by irreversible nucleophilic addition to oxidized matrix species (bold pathway, **Figure 10**). This nucleophilic addition might be assisted by a hydrophobic effect. For the benzylic thiols (e.g., FFT), our trials lead us to a new hypothesis. The inhibition of nucleophilic addition alone does not fully prevent the degradation of benzylic thiols; therefore, other pathways must take place in parallel. We suggest that a C-centered radical is formed in the presence of the coffee matrix and its reactive oxygen species such as $OH^{\bullet}$ (*9*). This species is thermodynamically favored over the thiyl radical $RS^{\bullet}$ and has a longer lifetime because it is stabilized by resonance. Therefore, it can survive in the coffee environment long enough to hit a neutral or a radical species from the matrix and thereby react irreversibly in a propagation or termination step. For the moment, this hypothesis is only supported by the stabilization

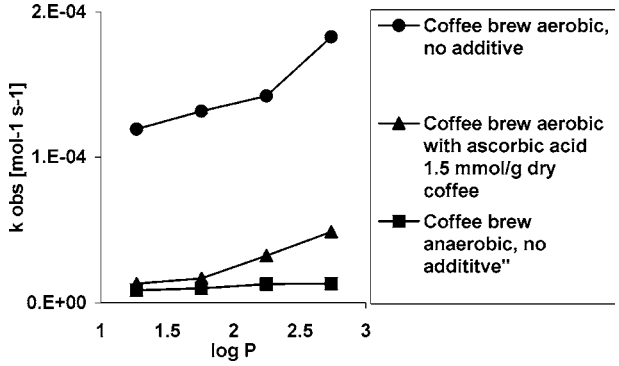

Figure 11. Correlation between observed reaction rate and log *P* of linear aliphatic thiols of increasing length.

observed in the presence of radical scavenger ascorbic acid. It needs further work such as EPR spectroscopy to be consolidated.

Hydrophobic Effect. Among the primary aliphatic thiols, a slight hydrophobic effect was noticed with increasing chain length of the homologous series. A direct correlation was found between the rate constant and the hydrophobicity in a series of homologous primary thiols (**Figure 11**). Therefore, weak physical interactions cannot be excluded at that point of the study. Hydrophobic interactions might also favor the approach of the thiol to the reactive site in the matrix and as a consequence slightly increase the rate of the nucleophilic additions.

This work provides an insight into the interactions between coffee matrix and coffee aroma. It proposes mechanistic schemes to explain the chemical degradation of volatile thiols in this complex environment. The key finding is that thiols, mainly responsible for the "roasty" and "burnt" notes (2-furfurylthiol, methanethiol, ethanethiol), disappear via two different mechanisms. Nucleophilic addition is the major pathway for thiol degradation. Addition occurs on oxidized species generated from the matrix in the presence of air. This mechanism prevails for aliphatic thiols (e.g., ethanethiol, methanethiol). Benzylic thiols (such as FFT) can react in parallel via another pathway that is slowed in the absence of oxygen and in the presence of radical scavenger ascorbic acid. This points to a radical mechanism, but further work is needed to support this hypothesis. A direct correlation between thiol hydrophobicity and the magnitude of the interactions was shown as well.

ABBREVIATIONS USED

t.s., total solid; EtSH, ethanethiol; PropSH, propanethiol; BuSH, butanethiol; 2BT, 2-butanethiol; 2M2P, 2-methyl-2-propanethiol; PentSH, pentanethiol; MMBF, 3-mercapto-3-methylbutylformate; BnSH, benzylthiol; PhSH, thiophenol; FFT, 2-furfurylthiol; ROS, reactive oxygen species; DTPA, diethylenetriaminepentaacetic acid.

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