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Interactions between Volatile and Nonvolatile Coffee Components. 1. Screening of Nonvolatile Components

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This study is the first of two publications that investigate the phenomena of coffee nonvolatiles interacting with coffee volatile compounds. The purpose was to identify which coffee nonvolatile(s) are responsible for the interactions observed between nonvolatile coffee brew constituents and thiols, sulfides, pyrroles, and diketones. The overall interaction of these compounds with coffee brews prepared with green coffee beans roasted at three different roasting levels (light, medium, and dark), purified nonvolatiles, and medium roasted coffee brew fractions (1% solids after 1 or 24 h) was measured using a headspace solid-phase microextraction technique. The dark roasted coffee brew was slightly more reactive toward the selected compounds than the light roasted coffee brew. Selected pure coffee constituents, such as caffeine, trigonelline, arabinogalactans, chlorogenic acid, and caffeic acid, showed few interactions with the coffee volatiles. Upon fractionation of medium roasted coffee brew by solid-phase extraction, dialysis, size exclusion chromatography, or anion exchange chromatography, characterization of each fraction, evaluation of the interactions with the aromas, and correlation between the chemical composition of the fractions and the magnitude of the interactions, the following general conclusions were drawn. (1) Low molecular weight and positively charged melanoidins present significant interactions. (2) Strong correlations were shown between the melanoidin and protein/peptide content, on one hand, and the extent of interactions, on the other hand ($R = 0.83-0.98$, depending on the volatile compound). (3) Chlorogenic acids and carbohydrates play a secondary role, because only weak correlations with the interactions were found in complex matrixes.

KEYWORDS: Coffee; headspace analysis; SPME; aroma stability; polysaccharides; melanoidins; chlorogenic acids; size exclusion chromatography; anion exchange chromatography; dialysis; SPE

INTRODUCTION

In addition to their physiological effects, coffee beverages are appreciated for their aroma and taste characteristics. Coffee aroma is the result of a complex balance of about 800 volatile compounds mainly formed during the roasting process. After being roasted, these compounds continue to be affected by environmental factors, their intrinsic instability, and interactions with matrix components. Interactions between volatile and nonvolatile compounds may be of physical (reversible) or chemical (reversible or irreversible) nature (*1*). Recently, Hofmann et al. (*2*, *3*) have observed that coffee aroma rapidly changes after preparation of the coffee brew. A strong decrease of the sulfury-roasty odor note was noticed at the same time as a decrease of 2-furfurylthiol, a key coffee odorant, in the headspace above coffee brew. Similar observations were made upon thermal processing of canned liquid coffee drinks (*4*). Our screening study performed with 21 coffee volatiles chosen for their chemical reactivity and/or their aroma impact indicated that thiols, sulfides, pyrroles, and diketones present significant interactions with coffee brew constituents, while aldehydes, esters, pyrazines, and guaiacols were not affected by 1% coffee brew upon 24 h storage (*5*).

The nonvolatile components of the coffee matrix play a major role in this instability. Indeed, model mixtures of coffee aroma without matrix are far more stable (*6*). Due to the complexity of coffee matrix, the relative contribution of its different components to the coffee aroma staling is still very poorly understood. Coffee nonvolatile components comprise carbohydrates, proteins/protein fragments, low molecular weight acids, chlorogenic acids, minerals, caffeine, trigonelline, lipids, and up to 30% of unknown molecules usually called melanoidins. Many of these classes of compounds have been shown to trap physically or chemically aroma components and could be involved in coffee aroma instability (*1*). Because only a few nonvolatile coffee compounds are commercially available, preparative fractionation is a necessary tool to screen the families of compounds responsible for coffee aroma instability. In the

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Table 1. Composition of Coffee Brews and Fractions (% w/w)

	yield [%]	ashes[a]	melanoidins[b]	carbohydrates[c]	proteins[c]	caffeine	chlorogenic acids	organic acids
				Brews				
light roasted[d]		14.5	33.6	11.8	6.0	7.7	12.4	13.9
medium roasted[d]		12.8	36.9	13.9	7.0	6.6	7.3	15.4
dark roasted[d]		12.6	43.7	15.3	5.0	5.8	1.8	15.7
				Dialysis (Medium Roasted Coffee Brew)				
permeate[d]	76.5	15.3	40.2	6.1	5.3	8.0	7.8	17.3
retentate[d]	19.5	4.5	53.5	31.8	8.6	0.2	0.5	0.9
				Size Exclusion (Medium Roasted Coffee Brew)				
SEC-A[d]	9.2	3.9	36.6	51.3	6.3	0.0	0.0	1.9
SEC-B	5.6	9.0	39.7	31.3	18.0	0.0	0.0	2.0
SEC-C	2.9	8.7	48.2	22.0	19.0	0.0	0.1	1.9
SEC-D[d]	9.9	15.7	35.2	12.0	9.1	0.0	0.0	28.0
SEC-E[d]	11.9	17.3	38.1	4.7	3.5	0.0	0.0	26.5
SEC-F[d]	17.3	28.1	35.7	2.8	6.3	0.0	0.0	22.8
SEC-G	20.8	7.0	49.7	2.6	3.1	25.6	7.6	3.4
SEC-H	4.1	10.4	22.7	1.3	1.1	7.2	51.7	5.2
SEC-I	7.6	11.5	50.2	1.5	1.0	0.4	30.1	5.3
SEC-J	1.3	6.1	75.5	3.6	1.2	0.5	11.2	1.8
SEC-K	1.2	8.5	79.7	3.7	2.0	0.3	3.4	2.5
				Anion Exchange (Medium Roasted Coffee Brew)				
AEC-A[d]	77.9	12.7	34.4	11.2	6.6	7.7	7.4	20.0
AEC-B[d]	4.1	1.4	55.2	3.4	8.9	0.4	18.1	12.5
AEC-C[d]	13.0	0.5	74.4	5.3	11.0	0	1.6	7.3
AEC-D[d]	1.5	0.5	84.8	2.2	9.2	0	0.4	2.8

[a] After combustion. [b] By difference. [c] After hydrolysis. [d] Samples used for the correlation study (**Table 3**).

past, coffee brew fractionation has been achieved by a succession of micro- or ultrafiltration steps followed by sensory evaluation and physicochemical characterization (*7*). Hofmann et al. (*2*, *3*) applied preparative scale size exclusion chromatography (SEC) and ultrafiltration to fractionate coffee brew. The highest thiol binding activity was observed with the low molecular weight fractions. Separations based on other physicochemical properties of the nonvolatile components, such as polarity and charge, would bring additional information.

This paper is the first of two publications, which seek greater understanding of the interactions that occur between volatile and nonvolatile coffee components. The goal was to determine which nonvolatile coffee components are mainly responsible for the coffee brew aroma degradation by a screening of the interactions between the sensitive volatile compounds (thiols, sulfide, pyrrole, and diketone) with different coffee matrixes. The coffee fractions studied were purified nonvolatile coffee components, dried coffee brews with three roasting levels, and coffee fractions separated by molecular weight, charge, or polarity. The effect of reconstituted coffee fractions on the headspace concentrations of the selected volatile compounds was monitored over time, and a statistical data analysis led to correlations between the composition of the coffee matrixes and the magnitude of the interactions. The second publication of this series will address the molecular mechanisms of the interactions between coffee matrix and sulfur volatiles.

MATERIALS AND METHODS

Purified Nonvolatile Coffee Components. Caffeine and caffeic acid were purchased from Fluka (Buchs, Switzerland), and 5-*O*-caffeoylquinic acid and trigonelline were from Sigma (Buchs, Switzerland). 1-Methylpyridinium iodide was obtained by synthesis (*8*). Arabinogalactans from larch wood were obtained from Sigma (Buchs, Switzerland), and those from acacia gum (Spraygum IRX 29345) were from Colloïdes Naturels International (Neuilly-sur-Seine, France).

Table 2. Solid-Phase Extraction of Medium Roasted Coffee Brew: Yields of Eluted (1) and Reversibly Retained (2) Fractions

type of solid-phase extraction	solid support	fraction 1 [%]	fraction 2 [%]
reversed phase apolar extraction	phenyl (Ph)	57	45
	cyclohexyl (CH)	60	36
	ethyl (C2)	59	44
	octyl (C8)	65	33
	octadecyl (C18)	52	51
polar extraction	cyanopropyl (CN)	93	7
	cellulose (Cell)	98	3
	polyamide (PA)	90	15
ion exchange	Dowex 50Wx8	43	17
	PS-benzenesulfonic acid (SCX)	79	23
	PS-trimethylaminopropyl (SAX)	79	26

Chemicals and solvents were obtained from Fluka (Buchs, Switzerland) or Merck (Dietikon, Switzerland) and used without further purification.

Coffee Samples. Coffee brew models were produced from a blend of Santos (Arabica) 50%, Colombia (Arabica) 30%, and Ivory Coast (Robusta) 20% with three roast levels, CTN 60 (dark), 85 (medium), and 110 (light), by countercurrent extraction 3 × 12 min at 120 °C. The extracts were spray-dried without preconcentration. The respective extraction yields were light roasted 21.5%, medium roasted 22.7%, and dark roasted 25.9%.

Coffee Fractionation. The dried brew from medium roasted coffee was separated into fractions based on physicochemical properties: size (dialysis and size exclusion chromatography), ionic charge (various ion exchangers), and polarity (reversed phase silica). Millipore water was used for all of the preparations described below. All of the fractions were concentrated at room temperature (RT), freeze-dried, further dried under high vacuum over P_2O_5, and stored at −20 °C until use.

*Rough Fractionation by Solid-Phase Extraction (**Table 2**).* Bondelut solid-phase extraction (SPE) cartridges were purchased from Varian (P. H. Stehelin, Basel, Switzerland) or prepared from bulk material. Solid phases were reversed phase (cyclohexyl, ethyl, octyl, octadecyl,

and phenyl), anion exchange (PS-trimethylaminopropyl), cation exchange (PS-benzenesulfonic acid), and polar (PS-cyanopropyl, cellulose from bulk, Serva, Chemie Brunschwig, Basel, Switzerland and polyamide-SC6 from bulk, Macherey-Nagel, Oensingen, Switzerland). Ten identical cartridges (1 mL, 100 mg) of each solid phase were conditioned on a Vac Elut Vacuum manifold according to suppliers' instructions. A 2% t.s. (total solids) solution of dried coffee brew was prepared, and 1 mL was eluted through each cartridge followed by 2 × 2 mL of H_2O per cartridge. The eluates were collected together and freeze-dried (fraction 1). The cartridges were further washed according to the solid phase: reversed phase and polar, CH_3CN (2 × 2 mL) and MeOH (2 × 2 mL); anion exchange (SAX), 1 M HCl (3 × 2 mL); cation exchange (SCX), 0.5 M NH_4OH (2 × 2 mL). The eluates were collected, evaporated to dryness under vacuum, taken up in water, and freeze-dried (fraction 2). Dowex 50Wx8 batchwise treatments were also performed. A 2% t.s. solution of coffee was prepared, and 100 mL was treated with 20 g of Dowex 50Wx8 (Fluka, Buchs, Switzerland) previously washed with 3 M HCl and Millipore H_2O to neutral pH. After 15 min agitation at room temperature (RT), the resin was filtered over a frit (por.3) and further washed with H_2O (150 mL) until complete decoloration of the filtrate. All filtrates were collected together and freeze-dried (fraction 1). Retained materials were partially recovered with 0.5 M NH_4OH (2 × 20 mL). The eluates were collected together, evaporated to dryness under vacuum, taken up in water, and freeze-dried (fraction 2).

Dialysis. Dried coffee brew (100 g in 400 mL of water) was dialyzed across a Spectra/Por membrane with a cutoff at 3500 Da (Sococochim, Lausanne, Switzerland) during 5 days at 4 °C (total of 10 washings). The permeate (low molecular weight fraction) and the retentate (high molecular weight fraction) were concentrated by RT evaporation under vacuum and freeze-dried. The yields were permeate 76.5% and retentate 19.5%.

Size Exclusion Chromatography (SEC). Size exclusion chromatography was adapted from Hofmann et al. (*2*). 2 g of dried coffee brew was dissolved in 15 mL of Millipore water and separated on a Sephadex G25 column 5 × 100 cm (Amersham Biosciences, Dübendorf, Switzerland) eluting with H_2O at a flow rate of 4 mL/min. Based on double wavelength detection (280, 325 nm), 11 fractions were isolated: SEC-A (mL 690−940, 9.2%), SEC-B (mL 940−1140, 5.6%), SEC-C (mL 1140−1280, 2.9%), SEC-D (mL 1280−1420, 9.9%), SEC-E (mL 1420−1540, 11.9%), SEC-F (mL 1540−1640, 17.3%), SEC-G (mL 1640−1920, 20.8%), SEC-H (mL 1920−2040, 4.1%), SEC-I (mL 2040−2400, 7.6%), SEC-J (mL 2400−2640, 1.3%), and SEC-K (mL 2640−3080, 1.2%) affording a total yield of 92%.

Anion Exchange Chromatography (AEC). DEAE-Sepharose-CL-6B (60 mL) (Fluka, Buchs, Switzerland) was suspended in 200 mL of degassed sodium acetate (AcONa 0.5 M). The stationary phase was allowed to settle, and the supernatant was poured off. The operation was repeated twice with AcONa and twice with degassed starting buffer pyridinium acetate (Pyr^+AcO^-, 0.05 M). The gel was transferred into a glass column (i.d. 2.5 cm) and equilibrated overnight by elution with 0.05 M Pyr^+AcO^- buffer. The coffee sample (5 g) was dissolved in the starting buffer (100 mL), added to the column, and eluted at 1.5 mL/min. The buffer concentration was increased stepwise (0.05, 0.1, 0.5, 1 M). The eluate from each step was collected in a single fraction and evaporated to dryness under vacuum. The oily residues were suspended in Millipore water, freeze-dried, and further dried over P_2O_5 under high vacuum for 24 h. The four fractions were isolated with respective yields of AEC-A, 77.9%; AEC-B, 0.1%; AEC-C, 13.0%; and AEC-D, 1.5%.

Characterization of Isolated Fractions. Total carbohydrates and total amino acids (both after hydrolysis), organic acids, chlorogenic acids, caffeine, trigonelline, and minerals after combustion were quantified directly with classical or previously described methods (*9*−*11*). Total carbohydrate results were expressed as anhydrosugars and total amino acids as peptides. The melanoidin content was obtained by difference and comprised all of the structurally unknown materials left after above quantifications. Analytical data are summarized in **Table 1**.

Coffee Fraction Oxidizability. The chemical reactivity toward oxidation was determined by amperometry. Sample preparation was carried out in a glovebox (Easy Box EB 80-1 spez., MecaPlex, Switzerland) with degassed acetate buffer (100 mM, pH 5). Stock solutions (0.1% w/vol.) were prepared from the corresponding coffee samples. After dilution to 0.01% w/vol. with acetate buffer and filtering over a syringe filter (0.45 μm), every solution was distributed into seven different 2 mL vials. A blank sample was prepared following the same procedure. Samples (5 μL) were injected on a HP-1090 HPLC instrument, equipped with an in-line frit followed by Teflon tubing (0.8 mm i.d. × 800 mm) to retard the peak. The area of the electrochemical signal was determined with a HP-1049A detector operating in the amperometric pretreat-mode with a glassy carbon working electrode and an Ag/AgCl reference electrode. Acetate buffer (100 mM, pH 5) was used as eluent at 1 mL/min. Samples were injected at seven increasing oxidation potentials from 200 to 800 mV, every 100 mV (one vial/potential). Peak areas were plotted against potentials.

Coffee Sample Electrophilic Reactivity. A 1.1% t.s. solution of the coffee sample was prepared and diluted 9:1 with a 10 mg/mL solution of *O*-phenylhydroxylamine (Fluka, Buchs, Switzerland). The sample (5 μL) was immediately analyzed by HPLC on a Nucleosil 100-5-C18 column (Macherey-Nagel, Oensingen, Switzerland, 250 mm × 4 mm, pore diameter 100 A, particle size 5 μm). The eluent system was Millipore water, 0.1% TFA, and CH_3CN at 1 mL/min. The consumption of the nucleophile *O*-phenylhydroxylamine was followed over time by HPLC with injections every 30 min during the first 3 h and then every 2 h. Results are expressed as mmol of reacted electrophile/g of dry coffee sample as a function of reaction time. The curves were fitted with the TC2D software using kinetic equations.

Measurement of the Interactions. Five coffee volatile aroma compounds were selected, representing the main chemical functions previously identified as reactive in the presence of coffee brew (*5*). They included two thiols, a sulfide, a pyrrole, and a diketone. They were purchased from Sigma-Aldrich (Buchs, Switzerland). Their final concentration was set to be in the linear range of the SPME fiber and at a ppm level: dimethyl sulfide (DMS, 1 mg/L), 2-furfurylthiol (FFT, 4 mg/mL), ethanethiol (EtSH, 2 mg/L), pyrrole (10 mg/L), 2,3-pentanedione (10 mg/L). The aroma compounds were mixed together and shown to be stable over 24 h at the selected concentrations, in acetate buffer (10 mM, pH 5.2).

Sample Preparation. The coffee matrix solution was designed to mimic a brew: 1% t.s. and pH 5.2. All of the solutions were prepared in acetate buffer (10 mM, pH 5.2). Solutions of coffee fractions and pure coffee components were prepared taking into account their ratio to the total coffee sample (yield of the fraction, **Table 1**). The purified nonvolatile components were used at the concentrations indicated in g/L: caffeine 0.5, chlorogenic acid 1.0, trigonelline 0.02, *N*-methylpyridinium iodide 0.03, larch wood arabinogalactan 3, and acacia gum arabinogalactan 3. The dried coffee brews were analyzed at 10 g/L. The dialysis fractions were analyzed at 2.0 g/L for the permeate and 7.8 g/L for the retentate. The size exclusion chromatography fractions were analyzed at 0.90 g/L for fraction SEC-A, 1.0 g/L for fraction SEC-D, 1.2 g/L for fraction SEC-E, and 1.7 g/L for fraction SEC-F. The anion exchange fractions were analyzed at 7.8 g/L for fraction AEC-A, 0.41 g/L for fraction AEC-B, 1.3 g/L for fraction AEC-C, and 0.15 g/L for fraction AEC-D. Aroma compounds and coffee matrix solutions were prepared at twice the final concentrations and then mixed (1:1) as described below. For each matrix to be tested, a blank volatile sample (2 mL aroma solution + 2 mL buffer) and a test sample (2 mL aroma solution + 2 mL coffee matrix solution) were prepared in 4 mL amber glass vials. The samples were stirred for 15 min at room temperature, and 800 μL was transferred into 2 mL amber silanized glass vials and equilibrated in the GC-autosampler at 25 °C for 24 h. For quick screening experiments (**Figures 2** and **3**), the coffee matrix was reconstituted at 1% t.s., and the matrix/aroma mixture was equilibrated for 1 h only before headspace analysis.

SPME-GC-MS Analysis. The headspace of the vials was sampled using a Varian CP-820 autosampler. A SPME fiber (PDMS/DVB; 65 μm, Supelco, Buchs, Switzerland) was inserted into the headspace and allowed to equilibrate for 1 min. An absorption time of 1 min was chosen so as to not saturate the fiber. To avoid a competition effect, the linearity of each compound was determined alone and in a mixture. The chosen concentrations were well under the upper limit of the linear

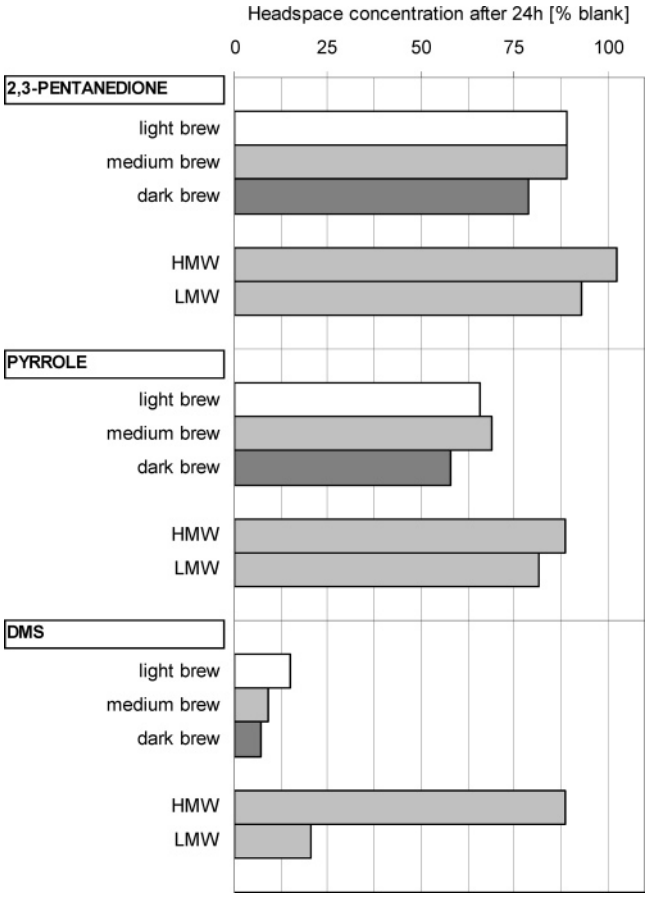

Figure 1. Comparison of the relative headspace concentrations of selected volatiles in coffee brew solutions and solutions containing dialysis fractions at their relative yield concentrations (acetate buffer 10 mM, pH 5.2, SPME-GC-MS measurement after 24 h). FFT = EtSH = 0 in all samples.

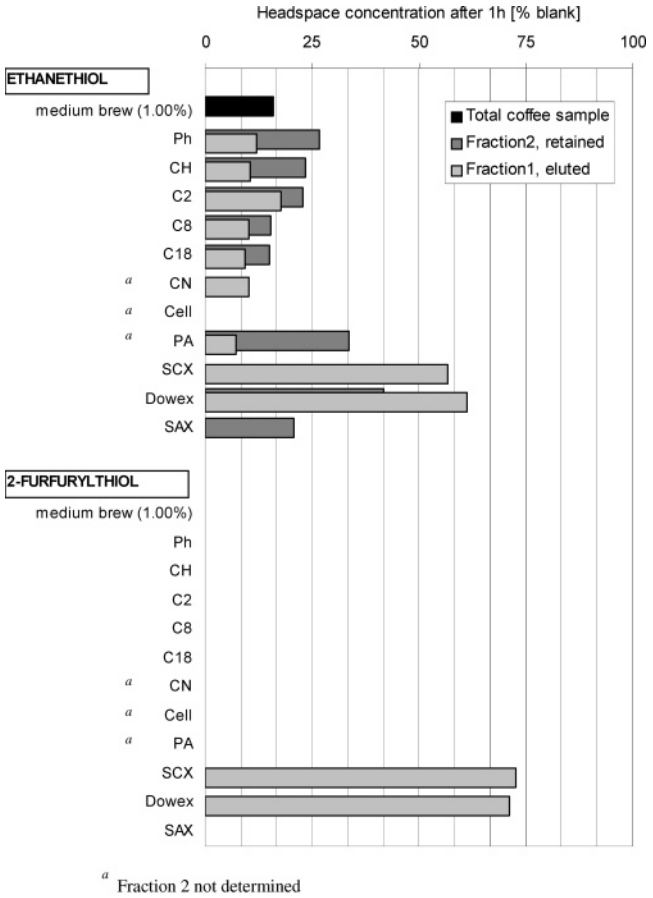

Figure 2. Comparison of the relative headspace concentrations (FFT and EtSH) in solutions containing SPE-fractions of medium roasted coffee brew at 1% t.s. (acetate buffer 10 mM, pH 5.2, SPME-GC-MS measurement after 1 h; fraction 1 is eluted material; fraction 2 is retained material).

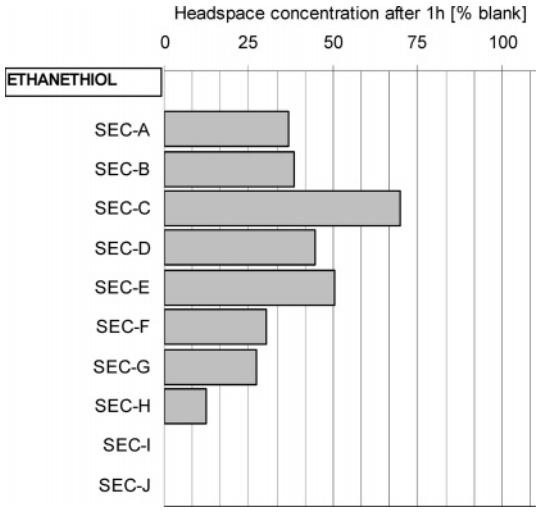

Figure 3. Comparison of the relative headspace concentrations (EtSH) in solutions containing SEC-fractions of medium roasted coffee brew at 1% t.s. (acetate buffer 10 mM, pH 5.2, SPME-GC-MS measurement after 1 h). FFT = 0 in all samples.

range. Aroma compounds were desorbed in the injector port of the GC for 5 min at 240 °C. During the first 3 min of desorption, the purge was off. GC separation was performed on a Hewlett-Packard 5973 gas spectrometer equipped 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 oven temperature was held at 35 °C for 3 min, and then programmed to 170 °C at 4 °C/min, then to 220 °C at 20 °C/min, and held at 220 °C for 10 min. Mass spectra were acquired in scan mode from 29 to 300 amu. The SPME-GC-MS peak area, that is, concentration of volatile compound in the presence of coffee solids, was expressed as a percentage relative to SPME-GC-MS peak area of the volatile compound in a blank volatile sample. Kinetic studies over 24 h were also performed. Preparation of samples was the same as described above. Vials containing blank flavored solution or coffee samples were put alternatively on the autosampler at time zero so that the headspace was sampled every 2 h in intact "aged" vials. The relative percentage was calculated from surface areas of blank and sample measured at the same sampling time.

Data Analysis. Relative headspace concentration was determined at least in duplicate for all samples. Depending on the samples, concentration was determined after 1, 24, or every 2 h between 0.5 and 18.5 h (kinetics). Duplicates were averaged, and means were visualized using bar charts (1 or 24 h), as well as scatter-plots (kinetics). One-way analysis of variance was applied to estimate an average pooled variance of duplicates. Based on this pooled variance, Fisher's least significant difference (LSD) was estimated on 5% significance level. The LSD was visualized on all figures as the intergrid distance. This allows a simple interpretation of the figures: samples with means differing by more than one intergrid distance can be declared as being significantly different on a 5% significance level. Pearson's correlation coefficient was estimated for relating the content of nonvolatile compounds to volatile losses after 24 h. Volatile losses are defined as 100% minus relative headspace concentration.

RESULTS

A preliminary study (5) on a medium roasted coffee brew and a set of 21 coffee aroma components belonging to different

chemical classes showed that only thiols, sulfides, pyrroles, and diketones were significantly affected after 24 h by the presence of coffee solution. The relative reactivity of coffee brews and fractions toward aroma components was therefore studied on a model volatile mixture comprised of dimethyl sulfide (DMS), 2-furfurylthiol (FFT), ethanethiol (EtSH), pyrrole, and 2,3-pentanedione. The screening was conducted on complete coffee extracts at various roasting levels, on commercially available or purified coffee components, and on coffee fractions obtained by various chromatographic techniques. Short time exposure (1 h) to the matrix was chosen to study the behavior of highly labile compounds such as the two thiols. A longer reaction time (24 h) was used to explore the reactivity of DMS, pyrrole, and 2,3-pentanedione.

Complete Coffee Extracts. Coffee brews (1% t.s.) from the same green coffee beans roasted at three different roasting levels all showed the same trends: the two thiols FFT and EtSH completely disappeared from the headspace in all three samples. The reactivity of the other test volatiles was: 2,3-pentanedione < pyrrole < DMS (**Figure 1**). The increase of the roasting degree resulted mainly in an increase of the melanoidin content and a decrease of the chlorogenic acids (**Table 1**). The magnitude of the interactions of 2,3-pentanedione, pyrrole, and DMS was slightly higher with the dark coffee brew as compared to the light coffee brew.

Purified Nonvolatile Coffee Components. To determine the relative importance of these compounds in the coffee aroma instability, the interactions were determined at the concentrations found in the coffee brew. Caffeine, trigonelline, 1-methylpyridinium iodide, and arabinogalactans from larch wood or acacia gum were studied and presented no significant interactions with the selected volatile compounds. Chlorogenic acid and caffeic acid decreased the headspace concentration of FFT (0% left after 24 h) but showed no effect on ethanethiol, 2,3-pentanedione, and pyrrole. The interaction between chlorogenic acid and FFT was completely suppressed in the absence of air (*5*).

To determine the main classes of nonvolatile compounds responsible for interactions with aroma, separations with various chromatographic techniques were conducted on the medium roasted coffee brew, and interactions between the corresponding fractions and the volatile compounds were evaluated.

Solid-Phase Extraction. SPE cartridges were used to perform a rough separation of coffee materials based on their physicochemical properties. The medium roasted coffee brew was separated by charge and polarity using various solid supports (**Table 2**). For each separation, fraction 1 refers to eluted material and fraction 2 the material retained by the solid support. Short-term interaction measurements were performed (1 h at 1% t.s.) with focus on the two sensitive thiols, FFT and EtSH (**Figure 2**). Reversed phase SPE (Ph, CH, C2, C8, and C18) retained increasing amounts of colored materials and caffeine with increasing hydrophobicity of the stationary phase. At 1% t.s., the eluted, polar material (fraction 1) was slightly more reactive toward EtSH than retained (apolar) material. FFT completely disappeared in all of these samples after just 1 h incubation. Polar extraction (CN, Cellulose & Polyamide) only retained a small fraction of the matrix. At 1% t.s., the eluted material (fraction 1) interacted with the two thiols to the same extent as the total matrix. Strong cation exchangers (sulfonic type: SCX, Dowex) retained some reactive species of the coffee sample. EtSH and FFT decreased less after 1 h incubation with the fraction 1 than with the total coffee sample at the same time and concentration. The results were similar when the coffee was passed through an SPE-cartridge and when it was treated batchwise at larger scale with bulk Dowex 50Wx8 resin. The retained materials were mainly colored compounds and caffeine but not chlorogenic acids. Treatment of the coffee sample with strong anion-exchange resin (SAX) retains the acidic small phenolic acids (chlorogenic, caffeic, and ferulic acids). The resulting neutral fraction (SAX-1) was more reactive than the total coffee sample toward the selected sulfur volatiles. This suggests a concentration of the reactive species in this matrix as compared to the starting material or an inhibition of the reactive species in the coffee brew as compared to the fraction obtained after SAX treatment.

This first screening step led us to the hypothesis that the reactive nonvolatile species are mainly polar, positively charged molecules. To go deeper into the identification of the coffee nonvolatiles involved in coffee aroma degradation, the medium roasted coffee brew was also fractionated by molecular size and charge.

Dialysis. The low molecular weight fraction (LMW < 3.5 kDa, 76%) contains most of the caffeine, the chlorogenic and organic acids, the low molecular weight melanoidins, and the ashes. The high molecular weight fraction (HMW > 3.5 kDa, 20%) is comprised of mainly polysaccharides and melanoidins (**Table 1**). Hofmann and Schieberle (*3*) have shown that low molecular weight coffee fractions present a higher reactivity toward thiols. Our results show that the dialysis permeate is also more reactive toward DMS. On the other hand, 2.3-pentanedione and pyrrole showed the same level of interactions with HMW and LMW. When the interactions of the permeate and the retentate were added, the resulting total interaction was in the same range as when the volatiles were exposed to the entire coffee (**Figure 1**).

Size Exclusion Chromatography. Based on double wavelength detection at 280 and 325 nm eleven fractions were isolated and characterized for their composition (SEC-A to SEC-K, **Table 1**). Some classes of compounds were well separated and found primarily in one single fraction (caffeine in G, phenols in H and I). Peptides/proteins were eluted mainly in HMW-fractions (A−F). Organic acids were distributed over the medium molecular weight fractions (D−F). Melanoidins (unknowns) were present in all of the fractions in variable amounts. As a function of the size exclusion chromatography, each fraction contains different sizes and structures of melanoidins (unknowns). As a first screening step, the interactions were measured after 1 h at a single concentration of 1% t.s. for each fraction (**Figure 3**). The most inert fractions were in the medium molecular weight range (especially fraction C). Between fractions C and I, the magnitude of the interactions increased with decreasing molecular weight.

Anion Exchange Chromatography. In this experiment, the nonvolatile compounds were separated according to their charge. In going from fraction AEC-A to AEC-D, the compounds go from neutral molecules to negatively charged molecules and go from light to dark color. All of the isolated fractions are mixtures, with some major variations of their relative compositions (**Table 1**). The samples were further characterized by their overall electrophilic reactivity and oxidizability with the goal to obtain qualitative information on the chemical reactivity. The fractions were reconstituted at real coffee concentration, and the interactions with the volatiles were measured over time. Fraction AEC-A was the most effective in decreasing volatile thiol headspace concentrations (**Figure 4**). The decay rate of EtSH was similar in this matrix and in the complete coffee sample. Fraction AEC-C, which only represents 13% of the starting material, also has a high potency of interaction. Fractions

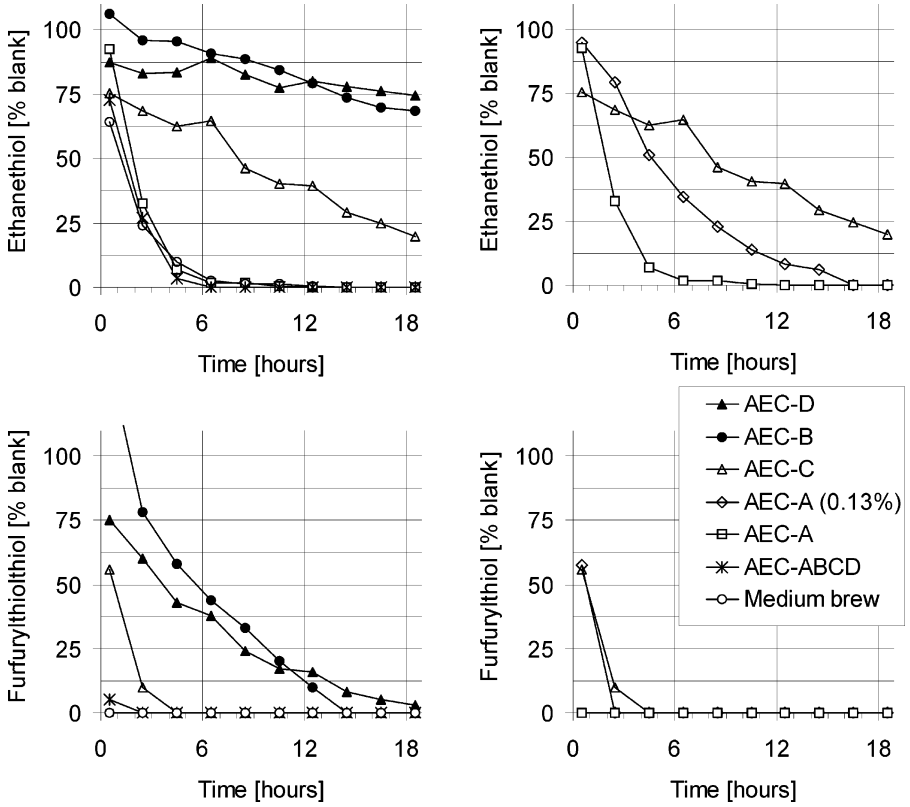

Figure 4. Comparison of the relative headspace concentrations (FFT and EtSH) in solutions containing AEC-fractions of medium roasted coffee brew at their relative yield concentrations (acetate buffer 10 mM, pH 5.2, SPME-GC-MS measurement every 2 h between 0.5 and 18.5 h).

AEC-B and AEC-D are less reactive. As observed with the complete matrixes, FFT was more affected than EtSH by all of the fractions tested. The electrophilic reactivity and oxidizability run in parallel with the thiol binding activity (**Figure 5**): fraction AEC-A shows the highest electrophilic reactivity and oxidizability, followed by fraction AEC-C. Interactions observed with the complete sample were recovered when the four fractions were recombined, and the kinetics were the same as those observed with the complete powder. Pyrrole was only decreased by fraction AEC-A, while 2,3-pentanedione was affected neither by the complete matrix nor by the fractions (data not shown).

The binding potency of fractions AEC-A and AEC-C was tested at the same concentration, that is, 0.13%, to determine if the higher retention of thiols observed with fraction AEC-A was due to its higher concentration or to the higher reactivity of the nonvolatile components. The results (**Figure 4**) indicate that fraction AEC-A is still more reactive than fraction AEC-C and therefore that the reactivity of the nonvolatile compounds present in fraction AEC-A is higher than that of fraction AEC-C. The different kinetics observed between the two fractions suggest that either the concentration of reactive species is different in the two fractions or that different mechanisms are occurring.

Correlations between Chemical Composition of Fractions and Interactions with Volatiles. Thirteen fractions were chosen to cover a large range of chemical compositions (**Table 1**). Pearson's correlation coefficient was estimated for relating the chemical composition of these matrixes to volatile losses measured after 24 h (**Table 3**; **Figure 6**). Due to the concentration factor, the contents of all chemical compounds are highly correlated. Melanoidins account for 34−85% w/w and for 0.01−0.45% t.s. It is the most important part in all fractions except SEC-A, and therefore correlated with the overall concentration ($r = 0.98$). Some components such as ash,

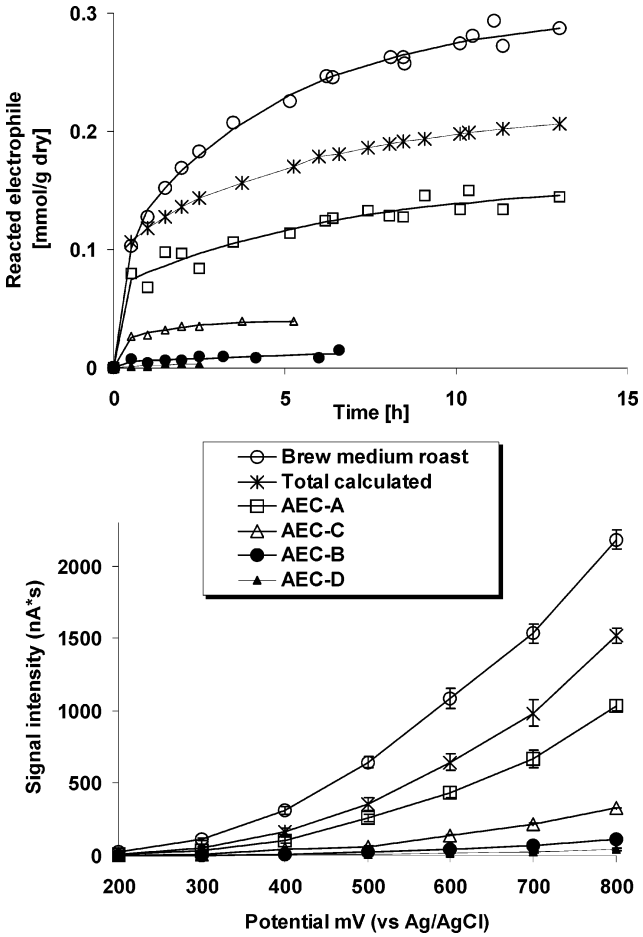

Figure 5. Electrophilic reactivity and oxidizability of AEC medium roasted coffee brew fractions normalized to the yield of individual fraction.

Table 3. Pearson's Correlation Coefficient Relating the Content of Nonvolatile Compounds to Volatile Losses after 24 h (13 Selected Samples)

	furfurylthiol	ethanethiol	pyrrole	DMS	2,3-pentanedione
overall concentration	0.39	0.80	0.95	0.97	0.88
melanoidins	0.41	0.83	0.97	0.98	0.92
ash	0.34	0.76	0.90	0.94	0.83
protein	0.41	0.83	0.92	0.95	0.81
caffeine	0.37	0.75	0.90	0.95	0.81
organic acids	0.30	0.75	0.88	0.95	0.83
carbohydrates	0.41	0.73	0.95	0.83	0.87
chlorogenic acids	0.33	0.63	0.72	0.79	0.56

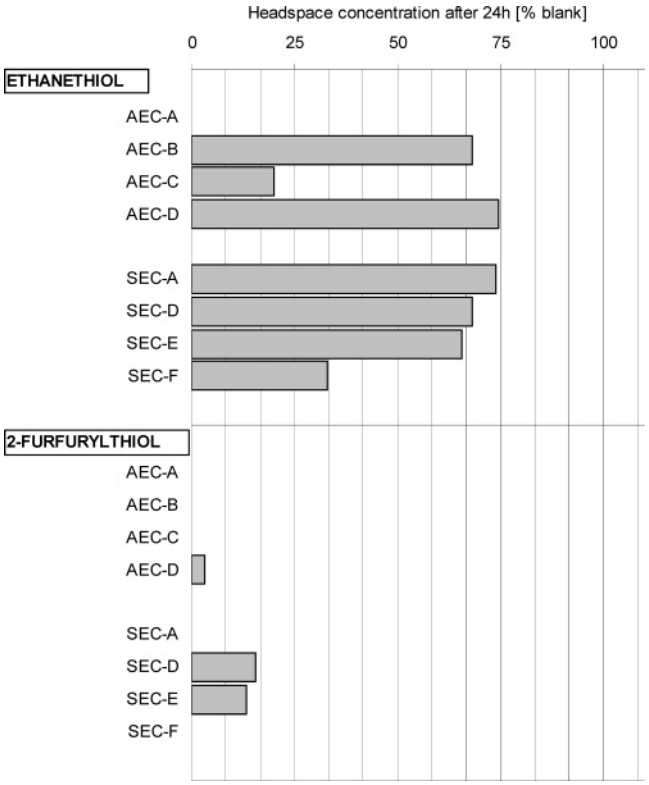

Figure 6. Comparison of the relative headspace concentrations (FFT and EtSH) in solutions containing selected SEC and AEC-fractions of medium roasted coffee brew at their relative yield concentrations (acetate buffer 10 mM, pH 5.2, SPME-GC-MS measurement after 24 h).

protein, caffeine, and organic acids are also highly correlated with the overall concentration ($r = 0.97$), while others are much less (chlorogenic acids, carbohydrates).

The correlation between content of individual nonvolatile components and volatile loss is always highest for melanoidins (**Table 3**). For FFT, losses were 100% after 24 h for all samples except three, which present less than 0.05% t.s. melanoidins. For ethanethiol, higher than 0.20% melanoidins lead to 100% loss after 24 h, whereas for lower than 0.20% melanoidin, content and losses are very highly correlated ($r = 0.96$). Pyrrole and DMS losses are highly correlated with melanoidin content ($r = 0.97$ and 0.98). No DMS loss is observed for fractions SEC-A and SEC-D. It is also lower than expected based on the melanoidin content for the dialysis retentate. It appears that these three fractions contain large amounts of carbohydrates, a class of compounds known for their chemical inertness. For 2,3-pentanedione, lower than 0.20% melanoidins led to negligible losses, whereas for higher than 0.20% melanoidin, content and losses are highly correlated ($r = 0.96$).

DISCUSSION

Influence of Volatile Compounds Classes and Structures. Thiols are the most affected by the nonvolatile coffee components. The decay curves (**Figure 4**) obey pseudo first-order rate laws and suggest that thiols chemically react with the coffee matrix. Thiols are known for their reactivity/instability in food systems. They are good substrates for oxidation, addition to electrophiles, and radical reactions. The coffee matrix has a large reservoir of reactive species for such reactions. In model systems, thiols bind covalently to Maillard-derived pyrazinium compounds (*3*) and also to oxidized chlorogenic acids (*12*). Both reactions occur via a nucleophilic addition. Hofmann et al. have shown that deuterated 2-furfurylthiol is incorporated into a high molecular weight fraction of coffee brew (*3*). Possible mechanistic pathways leading to thiol decrease in the presence of the coffee matrix is the subject of the second publication in this series and thus will not be discussed further here (*13*). DMS behaves similarly to EtSH. Its decay follows a pseudo-first-order rate law but with smaller rate constants than EtSH (*5*). Sulfides can be oxidized into sulfoxides and sulfones or add directly to coffee matrix components in a bimolecular chemical reaction. Pyrroles and diketones interact very slowly with matrix components (*5*). In many cases, the headspace concentrations as measured after 24 h incubation are not significantly different from the blank. This suggests physical interactions or slow chemical reactions. Pyrroles and diketones easily undergo acid-catalyzed polymerization (*6*).

Influence of Nonvolatile Coffee Components on Aroma Stability. The comparison of complete extracts or fractions tested at the same concentration shows that the magnitude and the rate of the interactions depend on the composition of the matrixes evaluated. Some general trends clearly stand out after statistical data treatment.

Chlorogenic Acids. In model systems, phenols only react with furfurylthiol. In the presence of O_2 and water, chlorogenic acids readily form reactive quinone species, and quinones are known to add to thiols (*14*). Furthermore, radicals such as OH· and HOO·, generated during the oxidation of chlorogenic acids or by Fenton reaction, can also react with thiols. In complete coffee matrixes, the real influence of chlorogenic acids on FFT instability is difficult to highlight. No correlation was observed between the chlorogenic acid content and the magnitude of the interactions. However, in the complete coffee brew, this reaction might be strongly accelerated by the presence of catalysts such as transition metals.

Carbohydrates. Based on literature data, some interactions were expected between high molecular weight carbohydrates (mannans and arabinogalactans) and aroma components (*15*). Our combined data, however, suggest that carbohydrates only contribute to a very small extent to the interactions observed between the whole coffee matrix and the sensitive aroma components tested. Indeed, no correlation was found between the carbohydrate content and the strength of the interactions.

Proteins. Proteins are known to interact with aroma compounds principally through hydrophobic interactions in hydrophobic pockets. During roasting, proteins are denatured and fragmented (*16*). Thereby, the tridimensional structure involved in hydrophobic interactions is destroyed as well. Interchange reactions between disulfide aroma compounds and the protein sulfhydryl groups have also been reported (*17*). Our combined data show only weak correlations between amino acid content (after hydrolysis) and the strength of the interactions except for pyrrole and DMS. Reversible hydrophobic interactions might be a part of the explanation. Thiol/disulfide interchange did not

take place. No decrease of dimethyl disulfide was observed (data not shown). During roasting, the sulfhydryl groups are consumed by the formation of volatile sulfur compounds and melanoidins. They are therefore no longer available for interchange reactions.

Melanoidins are usually defined very broadly as colored, nitrogen-rich, water soluble, or insoluble macromolecules showing large molecular weight distributions (*18*). This includes many different chemical structures and functionalities. In this work, we deliberately chose to quantify the melanoidins by difference after the determination of total sugars (after hydrolysis), total amino acids (after hydrolysis), organic acids, chlorogenic acids, caffeine, salts (ashes), and lipids. Green coffee contains no melanoidins and can be almost fully characterized (up to 95%) by a summation of the above components. Roasted coffee, on the other hand, contains 20−50% unknowns when submitted to the same analyses. These are the newly formed compounds due to the thermal treatment (Maillard reaction, pyrolysis, caramelization). This group of newly formed compounds is quite close to the definition of melanoidins given above. In our opinion, the calculation by difference is therefore the most sensible way to get an approximation of the amount of melanoidins in coffee. One should, however, keep in mind that this definition also includes low molecular weight structurally undefined and possibly colorless compounds. A correlation between the melanoidin content (as defined above) and the magnitude of the interactions was observed for sulfur compounds, 2,3-pentanedione and pyrrole: the higher is the melanoidin content, the stronger are the interactions (**Table 3**). Even though the structure of melanoidins is not yet well defined, fractionation shows that melanoidins represent different families of compounds from neutral slightly colored small molecules to charged and colored higher polymers. Several arguments point to the low molecular weight and charged molecules as being responsible for the interactions. Fractionation based on size showed that the low molecular weight fractions are more reactive than the high molecular weight fractions (**Figure 1**). Andriot et al. also showed that the retention of four volatile compounds by coffee melanoidins decreased when the degree of roasting increases and suggested that this result could be explained by a lower retention of volatiles by high-molecular weight melanoidins as compared to low-molecular weight melanoidins (*19*). SPE with strong cation exchangers resulted in decolorized matrixes with reduced activity toward sulfur volatiles (**Figure 2**). The positively charged "CROSSPY" structures identified by Hofmann et al. (*3*) as potential thiol binding molecules would be retained on such a stationary phase as well. Unfortunately, there is no method to quantify this family of compounds. A higher retention of isoamyl acetate with basic and acidic melanoidins has also been reported (*20*). Dipole−dipole or dipole−ion (NH_3^+, COO^-) interactions were proposed as mechanisms.

In this study, interactions between volatile and nonvolatile coffee components were studied by the relative change of headspace concentration between flavored buffer and flavored coffee solutions measured by SPME-GC-MS. The magnitude of the interactions depends on the volatile's chemical class. Thiols, especially FFT, present strong interactions with coffee nonvolatiles followed by sulfides and to a lower extent by pyrroles and diketones. Fractionation of coffee by anion exchange chromatography, dialysis, or size exclusion chromatography showed that melanoidins are the main nonvolatile coffee components involved in the interactions. The higher is the melanoidin content, the stronger are the interactions. Especially low molecular weight positively charged compounds were identified as the major players in the interactions between sulfur volatiles and the coffee matrix. Chlorogenic acid weakly interacts with FFT but not with a primary aliphatic thiol such as EtSH. Peptides/proteins might play a minor role in the matrix−volatile interactions. Polysaccharides were inert toward the investigated volatiles.

This work provides an insight into the interactions between coffee matrix and coffee aroma. A second publication (*13*) will focus on thiols, which are the most affected volatile compounds and also among the most important in terms of sensory perception of coffee aroma. In this second publication, phenomena observed at a macroscopic level will be examined from a mechanistic point of view.

ABBREVIATIONS USED

EtSH, ethanethiol; FFT, 2-furfurylthiol; DMS, dimethyl sulfide; t.s., total solid; AEC, anion exchange chromatography; SCX, strong cation exchange; SAX, strong anion exchange; SEC, size exclusion chromatography; cell, cellulose; PA, polyamide; CH, cyclohexyl; HMW, high molecular weight; LMW, low molecular weight.

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