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Changes in Key Odorants of Raw Coffee Beans during Storage under Defined Conditions

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During storage of raw coffee beans (green coffee) atypical odors may develop, which are suggested to influence the aroma of particularly the coffee beverage. To gain insight into the aroma compounds responsible for such odor changes, a comparative aroma extract dilution analysis was applied on unstored, raw Arabica coffee beans from Colombia (water content = 11.75%) and on the same beans with a water content of 13.5%, which were stored for 9 months at 40 °C. In combination with the flavor dilution (FD) factors, the results of the identification experiments showed strong increases in (*E*)-*β*-damascenone (cooked apple-like), 2-methoxy-4-vinylphenol (clove-like), and methyl 2-methyl- and methyl 3-methylbutanoate (fruity), whereas others, such as the earthy smelling 3-isopropyl-2-methoxypyrazine as well as 2-phenylethanol and 3-methoxyphenol, remained unchanged during storage. In addition, the previously unknown coffee odorant 2-methoxy-5-vinylphenol (intense smoky odor) increased significantly during storage. Quantitative measurements performed on raw coffee samples stored at various temperatures, water contents, and oxygen availabilities indicated that the significant increase of, in particular, the methyl esters of 2- and 3-methylbutanoic acid were responsible for the pronounced and fruity odor quality perceived in the stored green coffee, whereas the higher concentrations of 2-methoxy-4-vinylphenol and 2-methoxy-5-vinylphenol led to the more pronounced smoky, clove-like odor quality. On the basis of the results obtained, in particular the reduction of the water content in combination with lower temperatures can be suggested to avoid aroma changes in raw coffee beans caused by storage.

KEYWORDS: Raw coffee beans; off-odor; aroma extract dilution analysis; 2-methoxy-5-vinylphenol; stable isotope dilution analysis

INTRODUCTION

The characteristic set of aroma compounds present in coffee beverages is obtained as a result of three main processing steps: (i) the fermentation of the raw coffee beans, (ii) the roasting process, and (iii) the hot water extraction. Because the roasted beans bear the typical "roasted-coffee" aroma, numerous studies are available on the volatile fraction of roasted coffee beans, and to date ≈840 volatiles have been identified (*1*). However, previous studies using gas chromatography−olfactometry (GC-O) and odor activity value (OAV) calculations have shown that among them only 27 compounds are able to generate an odor quite similar to that of roasted Arabica coffee, when present in certain concentrations (*2*).

By contrast, studies on the volatiles evoking the earthy-green aroma of raw coffee beans are scarcely available. Vitzthum et al. (*3*) were the first to analyze the volatile fraction of raw coffee beans by means of GC-O. They identified four 3-alkyl-2-methoxypyrazines and concluded that, due to their low odor thresholds, 3-isopropyl-2-methoxy- and 3-isobutyl-2-methoxypyrazine are important aroma contributors. By quantification and calculation of OAVs, Czerny et al. (*4*) confirmed that the aroma of raw coffee is primarily caused by 3-isobutyl-2-methoxypyrazine. These authors also identified for the first time 2-methoxy-3,5-dimethylpyrazine as an important aroma-active compound in raw coffee.

For several reasons, dried raw coffee (green coffee) may be stored in the country of origin for up to 3 years before export and roasting. However, it is known by the coffee trade that coffee beverages prepared from roasted beans that have been stored longer in the unroasted state may show an off-note, and, in particular, coffee beans from Colombia are affected by this problem.

High storage temperatures and a higher water content have been reported as the major factors causing sensory changes in stored raw coffee (*5*). Also, an uncontrolled fermentation of the raw beans was reported to cause off-odors, for example, by an increase in ethyl 2-methylbutanoate and ethyl 3-methylbutanoate (*6*). In addition, these authors also detected ethyl cyclohexanoate in overfermented beans, and already at low concentrations this

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ester caused a fruity, silage-like off-flavor when added to a coffee beverage. Lopez et al. (*7*) found that properly fermented Arabica coffee from Colombia with a water content of 10−12%, which was stored for 9 months at 20 °C, kept a good beverage quality. However, in samples with a higher humidity molds proliferated in the last months of storage, leading to a strong "mold-like" taste in the beverage (*7*).

Illy and Viany (*8*) detected an off-flavor reminiscent of rotten fish in immature green beans, and Full et al. (*9*) later suggested 4-heptenal as a key odorant for this aroma defect. On the other hand, Becker et al. (*10*) had reported that a pea-like off-flavor in roasted East African coffee was caused by unusual high concentrations of 3-isopropyl-2-methoxypyrazine. This off-flavor, which is also called "potato taste", is obviously caused by insects (especially by the variegated coffee bug), because methoxypyrazine-producing bacteria can penetrate into hurt coffee cherries (*11*). Methoxypyrazines did not decrease during coffee roasting (*4*), so it seems probable that in higher concentrations these might affect the aroma of the coffee beverage. In addition, also the oxidation of coffee lipids and the generation of (*E*)-2-nonenal has been proposed as a source of off-odors in coffee beverages (*12*), and it was shown that at a level of 8 μg/L of this compound caused a woody off-note.

Because the literature survey indicates that the storage conditions of raw beans seemed to be an important parameter in off-odor development in coffee beverages, the present study was aimed at (i) characterizing the key odorants in authentic fresh raw coffee beans by application of the odor activity value concept and (ii) monitoring changes in the concentrations of selected odorants occurring during storage of the same batch of coffee beans under defined conditions.

MATERIALS AND METHODS

Coffee Samples. Raw Arabica coffee beans from Colombia were provided by a German coffee trading company. The time between raw coffee production and the storage experiments was about 3 weaks. To adjust defined water contents, aliquots of the raw beans with a water content of 11.75% (30 kg) were carefully dried in hot air to 6.2%. To reach a water content of 13.5%, water (10 L) was added to another aliquot (30 kg) of the raw coffee and the suspension was stirred at 45 °C for 20 min. No measurable extraction of green coffee constituents occurred during this short period.

To maintain a defined oxygen atmosphere, aliquots (2 kg) of the three coffee samples were kept in airtight alumina bags at 2 or 20% oxygen atmosphere, respectively. The total headspace volume was 200 mL. The sealed bags were stored at either 12 or 40 °C, respectively.

Thus, the following green coffee samples varying in one parameter were obtained: series A with 13.5% water content included samples stored at 20% oxygen and 12 °C, 2% oxygen and 40 °C, and 20% oxygen and 40 °C. The respective B series with 11.75% water content and the C series with 6.2% water content were stored under the same conditions. Aliquots of the fresh raw coffee beans were stored at −30 °C as the reference. Storage conditions were chosen according to advice by the coffee trade.

Chemicals: Reference Aroma Compounds. The following compounds were obtained from commercial sources given in parentheses: (*R*/*S*)-methyl 2-methylbutanoate, (*R*/*S*)-ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, hexanal, 2-ethyl-3,5-dimethylpyrazine, 2-methoxy-3-isopropylpyrazine, 3-(methylthio)propanal (methional), (*E*)-2-nonenal, 2-methoxy-3-isobutylpyrazine, (*R*/*S*)-2-methylbutanoic acid, 3-methylbutanoic acid, (*E*,*E*)-2,4-nonadienal, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDF), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (3-HDF), 4-methoxy-3-hydroxybenzaldehyde, 3-methoxyphenol, and 4-hydroxy-3-methoxybenzaldehyde (vanillin) (Aldrich, Sigma-Adrich Chemie, Taufkirchen, Germany); acetic acid (Merck, Darmstadt, Germany); 2-phenylethanol (Fluka, Sigma-Aldrich Chemie, Taufkirchen, Germany); methyl 3-methylbutanoate and 2-methoxy-4-vinylphenol (4-vinylguaiacol) (Lancaster, Mühlheim/Main, Germany). (*E*)-β-Damascenone was a gift from Symrise (Holzminden, Germany). 2-Methyl-3-(methyldithio)furan was synthesized according to the method given in ref *13*.

Syntheses. *2-Methoxy-5-vinylphenol.* A suspension of an instant-ylide mixture of methyltriphenylphosphonium bromide with sodium amide (1.5 g = 3.6 mmol of methyl triphenylphosphoniumbromide) (*14*) and dry diethyl ether (10 mL) was vigorously stirred for 15 min in a round-bottom flask covered with a piece of cotton wool. 4-Methoxy-3-hydroxybenzaldehyde (0.2 g, 1.3 mmol) dissolved in dry diethyl ether (2 mL) was added, and the mixture was stirred for 45 min at room temperature. After the addition of a saturated aqueous NH_4Cl solution (50 mL), the organic phase was separated and the aqueous phase was extracted with diethyl ether (2 × 20 mL). The organic layers were combined, dried over anhydrous Na_2SO_4, and evaporated to dryness at 40 °C. The crude material was taken up in dichloromethane (1 mL) and purified by flash chromatography (glass column, 40 cm × 2.5 cm) on silica gel 60 (particle size = 0.035−0.070 mm) by stepwise elution (100 mL each) with solvent mixtures (v/v by volume) of increasing polarity: A, pentane; B, pentane/dichloromethane, 95:5; C, pentane/dichloromethane, 90:10; D, pentane/dichloromethane, 85:15; E, pentane/dichloromethane, 80:20; F, pentane/dichloromethane, 75:25; G, pentane/dichloromethane, 70:30; H, pentane/dichloromethane, 60:40; and I, pentane/dichloromethane, 50:50. The target compound was eluted in fractions G and H. After removal of the solvent at 40 °C, 155 mg (yield = 78%) of a white powder was obtained (*15*): MS-CI, *m*/*z* (%) 151 (100), 137 (18), 152 (9); MS-EI, *m*/*z* (%) 135 (100), 150 (92), 107 (25), 77 (18), 136 (7), 151 (7), 79 (6), 51 (5), 78 (5); ^{1}H NMR (360 MHz, d_4-methanol, TMS), δ 3.84 (s, 3H), 5.05 (dd, J = 1.2 and 10.9 Hz, 1Hβ), 5.56 (dd, J = 1.2 and 17.7 Hz, 1Hα), 6.58 (dd, J = 10.9 Hz and J = 17.7, 1H), 6.83−6.84 (m, 2H), 6.91−6.92 (m, 1H).

Isotopically Labeled Compounds: [$^2H_3$]-2-Methoxy-4-vinylphenol. Starting from [$^2H_3$]-4-hydroxy-3-methoxybenzaldehyde (100 mg), prepared as reported in ref *16*, [$^2H_3$]-2-methoxy-4-vinylphenol was synthesized following the procedure described above for the unlabeled 2-methoxy-5-vinylphenol. Yield = 0.1 g (100%). The MS-EI and MS-CI data were in agreement with data reported in ref *16*.

The following isotopically labeled compounds were prepared as described previously: [$^2H_3$]-methyl 2-methylbutanoate (*17*), [$^2H_2$]-(*E*,*E*)-2,6-nonadienal (*16*), [$^2H_{4-6}$]-(*E*)-β-damascenone (*18*), [$^2H_2$]-(*E*)-2-nonenal (*19*), [$^2H_3$]-2-methoxy-3-isopropylpyrazine (*20*), [$^2H_3$]-ethyl 2-methylbutanoate (*21*), [$^2H_3$]-ethyl 3-methylbutanoate (*21*).

Dichloromethane, *n*-pentane, ethanol (Lichrosolv), dry diethyl ether, cellulose, anhydrous sodium sulfate, sodium hydrogen carbonate, sodium chloride p.A., hydrochloric acid p.A. (32%), and ammonium chloride p.A. were purchased from Merck. Dichloromethane and pentane were freshly distilled before use. Silica gel 60 (particle size = 0.035−0.070 mm) and the instant-ylide (methyltriphenylphosphonium bromide with sodium amide) were from Fluka (Sigma-Aldrich Chemie).

Isolation and Fractionation of Volatiles. Raw coffee beans (30 g) were frozen with liquid nitrogen and powdered in a ultracentrifugal mill (type ZM1; Retsch, Haan, Germany; diameter of the pores = 2 mm). The powder was extracted by stirring with dichloromethane (200 mL) at ambient temperature for 1 h. After filtration, the residue was extracted twice with dichloromethane (100 mL each) for another 90 min. The extracts were combined and concentrated to 100 mL using a Vigreux column (50 cm × 1 cm). After concentration to 100 mL, the volatiles were isolated by SAFE distillation (*22*).

The distillate was separated into the acidic and the neutral-basic volatiles as reported previously (*23*). The fractions containing either the neutral and basic volatiles (NBF) or the acidic volatiles (AF) were dried over anhydrous sodium sulfate and concentrated to 0.3 mL using a Vigreux column (50 cm × 1 cm) followed by microdistillation (*24*). For compound identification, the NBF fraction was further separated by column chromatography on silica gel (*25*).

High-Resolution Gas Chromatography−Olfactometry (HRGC-O); Mass Spectrometry. For HRGC-O a gas chromatograph type Trace GC 2000 series (Thermo Finnigan, Egelsbach, Germany) was used. Helium at a pressure of 110 kPa served as the carrier gas. Samples were applied by cold-on-column injection onto capillaries DB-FFAP

Table 1. Selected Ions (*m*/*z*) and Response Factors Used in the Stable Isotope Dilution Assays

compound	analyte (*m*/*z*)	internal standard (*m*/*z*)	response factor[a]
methyl 2- and 3-methylbutanoate[b]	117	120	0.95
(*E*,*E*)-2,4-nonadienal	139	141	0.81
2-methoxy-4-vinylphenol	151	154	1.04
(*E*)-β-damascenone	191	195–197	0.96
2-methoxy-5-vinylphenol[c]	151	154	1.00
(*E*)-2-nonenal	141	143	0.70
2-methoxy-3-isopropylpyrazine	153	156	0.87
ethyl 2-methylbutanoate	131	134	0.90
ethyl 3-methylbutanoate	131	134	0.90

[a] Response factors were determined by analyzing known mixtures of the analyte and the respective internal standard. [b] Isomers were not separated during GC; [$^2H_3$]methyl 2-methylbutanoate was used as internal standard to quantify the sum of both isomers. [c] [$^2H_3$]-2-Methoxy-4-vinylphenol was used as internal standard.

or DB-5 [both 30 m, 0.32 mm i.d., 0.24 μm film thickness; DB-FFAP (J&W Scientific, Agilent Technologies, Waldbronn, Germany), DB-5 (CP SIL 8CB) (Chrompack, Darmstadt, Germany)] using the equipment described (*23*). Samples (1 μL) were injected at an oven temperature of 40 °C. After 2 min, the temperature was raised by 6 °C/min to 240 °C. The final temperature was held for 10 min. Retention indices (RI) were calculated from the retention times of a homologous series of *n*-alkanes by linear interpolation.

Mass spectra were recorded using a gas chromatograph 5890 series II (Hewlett-Packard, Heilbronn, Germany) connected to a sector field mass spectrometer type MAT 95 S (Finnigan, Bremen, Germany). Mass spectra in the electron ionization mode (MS-EI) were recorded at 70 eV ionization energy and mass spectra in the chemical ionization mode (MS-CI) at 115 eV using isobutane as the reactant gas.

Aroma Extract Dilution Analysis (AEDA). For AEDA, AF and NBF were stepwise diluted (1:1) with dichloromethane. Dilutions 1:2, 1:4, 1:8, 1:16, etc., of the original extracts were each evaluated by HRGC-O. Dilution was continued until no odorant could be detected. Each odorant was, thus, given a flavor dilution (FD) factor representing the last dilution in which the odorant was detectable (*26*). The AEDA was performed by three panelists.

Quantitation by Stable Isotope Dilution Assays in Combination with GC-MS. Raw coffee beans were powdered as described above. The coffee powder [5−25 g, depending on the concentration of the respective odorant (determined in preliminary experiments)] was suspended in dichloromethane (100−400 mL), and the labeled internal standards, dissolved in dichloromethane, were added. The amount of the respective internal standard was chosen in a similar concentration range as compared to the analyte. After equilibration overnight, the volatiles and the internal standards were isolated by SAFE distillation, and the solution was concentrated as described above.

The distillates were analyzed by two-dimensional HRGC-MS using an HRGC Trace GC 2000 series (Thermo Finnigan) coupled with an HRGC CP 3800 (Varian, Darmstadt, Germany) and a Combi PAL autosampler (CTC Analytics, Zwingen, Swizerland). Samples were injected by the cold-on-column technique. The separation of the extract in the first dimension was achieved on the FFAP or the SE-54 column (for separation of methyl 2- and 3-methylbutanoate), respectively. The elution range containing the respective odorant and the internal standard was quantitatively transferred into a cold trap (SGE, Darmstadt, Germany) using the moving capillary stream switching system (Thermo Finnigan). The trapped material was then transferred onto the second column (OV-1701) and, finally, into an ion trap mass spectrometer (Saturn 2000, Varian) running in the CI mode with methanol as the reactant gas (ionization energy = 110 eV). From the mass chromatograms, areas under the peaks at the respective mass traces were recorded, and concentrations were determined using the intensities of the ions of the unlabeled and of the labeled compound given in **Table 1**.

Aroma Profile Analysis. The powdered raw coffee beans were orthonasally evaluated by 15 trained panelists, who were asked to rank the intensity of 13 odor qualities in the overall aroma of raw coffee, as determined in preliminary tests. The respective odor intensities were rated using a scale of 0, 0.5, 1, 1.5, etc., with 0 (not perceivable), 1 (weak), 2 (significant), and 3 (strong).

Panelists recruited from the German Research Center of Food Chemistry underwent training for odor qualities and odor intensities using different concentrations of the respective odorants in water. Odor qualities were specified using the following reference compounds: hexanal (green), ethyl 2-methylbutanoate (fruity), 3-isopropyl-2-methoxypyrazine (pea-like), 2-ethyl-3,5-dimethylpyrazine (earthy), 3-isobutyl-2-methoxypyrazine (bell-pepper-like), 4-vinyl-2-methoxyphenol (clove-like), (*E*)-β-damascenone (cooked apple-like), (*E*)-2-nonenal (fatty), acetic acid (vinegar-like), methional (cooked potato-like), 3-methylbutanoic acid (sweaty), and 3-hydroxy-4,5-dimethyl-3(2*H*)-furanone (seasoning-like).

Odor Thresholds. First, the purity of odorants was checked by HRGC-O before use. As matrix for the determination of odor thresholds, tap water or sunflower oil ("Vitador", Lidl, Neckarsulm, Germany), respectively, was used. To prepare the reference solutions, a defined amount of the odorant, dissolved in 0.1 mL of ethanol, was added to either water (1 L) or oil (500 mL). After intense shaking, the samples were diluted stepwise 1:3 (v/v) with the respective matrix. Each test sample was then orthonasally evaluated in a series of triangular tests against two blank samples (odorless matrix). For this purpose, the samples (10 mL of water or 10 mL of oil) were filled into cylindrical ground neck glasses (height = 7 cm; i.d. = 3.5 cm) with caps. The triangular tests were presented in order of increasing concentrations of the test samples. Evaluations were performed in a sensory room.

The odor threshold values in water or oil were determined by 19 trained panelists, who judged whether they could still smell the odorant (recognition threshold) as compared to the odorless matrix or just perceived a difference (detection threshold).

Odor thresholds in cellulose were determined by adding a defined amount of the odorant dissolved in ethanol (0.2 mL) to cellulose (250 g; Merck). The mixture was homogenized by intense shaking for 15 min and then stepwise (1:3, w/w) diluted with cellulose. The samples (4 g each) were presented and evaluated as described above. For the blank samples, odorless cellulose was used. All odor thresholds were calculated according to the method described in ref *27*.

Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR). ^{1}H NMR spectra were recorded with a Bruker AM 360 spectrometer operating at 360 MHz. The respective compound was dissolved in [$^2H_4$]-methanol containing tetramethylsilane (TMS) as internal standard.

RESULTS AND DISCUSSION

Identification of Odor-Active Constituents in Unstored Raw Coffee Beans. In a first experiment the volatile fraction from fresh, unstored raw coffee beans (REF) was isolated by solvent extraction followed by SAFE distillation. The distillate elicited the overall aroma profile of freshly ground raw coffee, in which odor attributes such as pea-like, green, and earthy predominated when aliquots of the extract were evaluated by a sensory panel using strips of filter paper.

Before application of the AEDA, acidic compounds were separated from the volatile fraction by treatment with aqueous sodium hydrogen carbonate. Preliminary experiments had shown that this separation step was necessary to avoid interference with predominant acidic compounds during GC-O. By application of GC-O, 13 odor active areas were detected in the NBF fraction and 3 in the acidic fraction. The compounds elicited intense earthy, pea-like, bell pepper-like, or flowery odor qualities. To characterize the odorants responsible for these odor notes, first, their retention indices on two columns of different polarities and their odor qualities were compared to data available in an in-house database prepared by analyzing about 600 volatiles known as food odorants.

To obtain sufficient amounts for the identification experiments, the NBF was isolated from 500 g of raw beans and separated by column chromatography on silica. In the respective

Table 2. Most Odor-Active (FD Factor ≥ 32) Volatile Constituents in Unstored (REF) and Stored Raw Coffee Beans (Sample A, 20%–40 °C)

no.	odorant[c]	odor quality[d]	RI[a] on FFAP	RI[a] on DB-5	FD in[b] REF	FD in[b] A	earlier identified as volatile in raw coffee[e]
1	methyl 2- and 3-methylbutanoate	fruity	1022	770	1	512	*32*
2	ethyl 2-methylbutanoate	fruity	1043	850	32	8	*28*
3	ethyl 3-methylbutanoate	fruity	1064	854	64	16	*28*
4	hexanal	grassy, green	1074	800	32	16	*28*
5	2-methoxy-3-isopropylpyrazine	earthy, pea-like	1418	1093	256	32	*3*
6	3-(methylthio)propanal (methional)	cooked potato-like	1444	902	64	128	*29*
7	2-methoxy-3-isobutylpyrazine	bell pepper-like	1511	1179	128	64	*3*
8	2-methyl-3-(methyldithio)furan[f]	meaty	1651	1169	32	256	
9	2- and 3-methylbutanoic acid[g]	sweaty	1657	867	256	512	*29*
10	(*E,E*)-*2,4-nonadienal*	fatty, green	1696	1210	32	<1	*33*
11	unknown	mint-like	1715	1242	32	32	
12	(*E*)-*β*-damascenone	cooked apple	1800	1385	16	256	*34*
13	2-phenylethanol	flowery	1900	1110	128	256	*33*
14	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (4-HDF)[g]	caramel-like	2024	1077	8	32	
15	3-methoxyphenol	phenolic	2086	1346	64	128	
16	2-methoxy-4-vinylphenol	clove-like	2191	1311	32	1024	*3*
17	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (sotolon)[g]	seasoning-like	2191	1108	64	64	*4*
18	2-methoxy-5-vinylphenol	smoky	2223	1329	<1	1024	
19	4-hydroxy-3-methoxybenzaldehyde (vanillin)	vanilla-like	2564	1396	32	64	*34*

[a] RI, linear retention index. [b] FD, flavor dilution factor. [c] The compound was identified by comparing it with the reference substance on the basis of the following criteria: retention index (RI) on the capillaries detailed in the table, mass spectra obtained by MS-EI and MS-CI, and odor quality as well as odor intensity perceived at the sniffing port. The odor intensity was estimated by checking the intensity of the FID-signal vs the odor perception in comparison to the reference compound. [d] Odor quality perceived at the sniffing port. [e] Reference number. [f] The MS signals were too weak for an unequivocal interpretation. The compound was identified on the basis of the remaining criteria given in footnote *a*. [g] The compound was detected in the acidic fraction.

fractions of increasing polarity, the odor-active compounds were detected by GC-O and analyzed by mass spectrometry in the CI or EI mode, respectively. The mass spectra, retention indices, and odor quality were finally compared to data obtained for the respective reference compounds. A very important criterion for identity is, however, the odor threshold of a given compound, because in the case of two coeluting volatiles present in different concentrations, the major compound might give the mass spectrum, whereas the minor one is detected by its odor. To avoid erroneous results, it is important to check that an analyte elicits the odor quality at the same concentration level as the reference compound.

The results of the identification experiments showed the highest FD factors for 2-methoxy-3-isopropylpyrazine (**5**, earthy, pea-like), 2-methoxy-3-isobutylpyrazine (**7**, bell pepper-like), and 2-phenylethanol (**13**, flowery) in the NBF of the unstored green coffee (REF; **Table 2**). Odorants with somewhat lower FD factors were characterized as ethyl 2-methylbutanoate (**2**, fruity), ethyl 3-methylbutanoate (**3**, fruity), hexanal (**4**, grassy, green), methional (**6**, cooked potato-like), 2-methyl-(3-methyldithio)furan (**8**, meaty), (*E,E*)-2,4-nonadienal (**10**, fatty), 3-methoxyphenol (**15**, phenolic), 2-methoxy-4-vinylphenol (**16**, clove-like), and vanillin (**19**, vanilla-like). The most odor-active compounds in the acidic fraction were identified as 2- and 3-methylbutanoic acid (**9**, sweaty), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**14**), and 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**17**, seasoning-like). A comparison with the available literature (**Table 2**) indicates that, in particular, 2-methyl-3-(methyldithio)furan (**8**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, and 3-methoxyphenol (**15**) were identified for the first time in raw coffee beans.

To gain a first insight into changes in key odorants induced by storage, raw coffee beans of this same batch were stored under quite drastic conditions (9 months at 40 °C under an atmosphere of 20% oxygen; 13.5% water content). An evaluation of the overall aroma profile of these beans revealed a very pronounced increase in smoky, clove-like aroma notes (**Figure 1**). Additionally the panelists characterized the stored coffee beans to be more fruity, sweaty, spicy, and baked apple-like, whereas the green, peasy, and earthy impressions were rated lower (*26*).

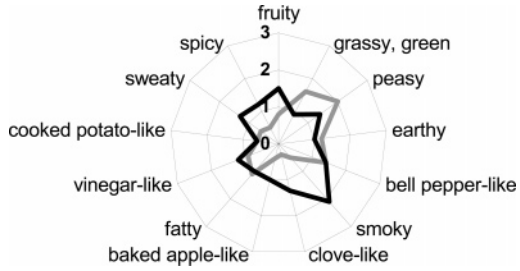

Figure 1. Aroma profiles of unstored raw coffee beans (REF; gray line) and coffee beans with a water content of 13.5% stored for 9 months at 40 °C under an atmosphere of 20% oxygen (A, 20%–40 °C; black line).

To elucidate the aroma changes on a molecular level, the volatile fraction was isolated from this sample stored for 9 months at 40 °C in an atmosphere of 20% oxygen (13.5% water) assigned as A, 20%−40 °C. The differences in the intensities of the most odor-active compounds, as compared to the unstored sample (REF), were analyzed by application of a comparative AEDA (*26*).

In the stored sample, two compounds eliciting an intense smoky odor showed the highest FD factor, namely, 2-methoxy-4-vinylphenol (**16**; **Table 2**) and compound **18**. The latter compound showed a mass spectrum very similar to that of 2-methoxy-4-vinylphenol (**Figure 2**) and, thus, its structure was assumed to be a positional isomer. By synthesis of 2-methoxy-5-vinylphenol and a comparison of retention indices and mass spectra, this structure was confirmed for compound **18**. In the unstored sample, both phenols either showed a much lower FD factor (**16**) or were even not detected (**18**).

2-Methoxy-5-vinylphenol was previously unknown in either raw or roasted coffee, but has been described as volatile constituent in, for example, cloudberry (*30*) or marijuana smoke (*31*). The analytical differentiation from 2-methoxy-4-vinylphenol is possible only on the basis of the differences in the

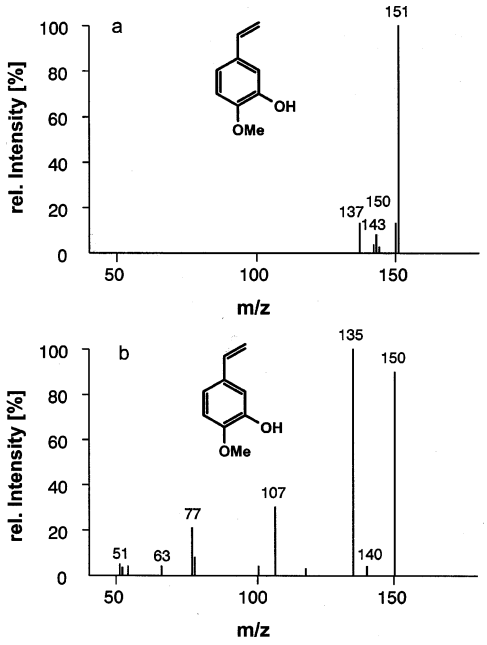

Figure 2. Mass spectra of compound **18** detected in sample A, 20%–40 °C: (**a**) MS-CI; (**b**) MS-EI.

Table 3. Comparison of Orthonasal Detection Thresholds (ODT) and Orthonasal Recognition Thresholds (ORT) of 2-Methoxy-4-vinyl- and 2-Methoxy-5-vinylphenol

	odor threshold in					
	water (μg/L)		oil (μg/L)		cellulose (μg/kg)	
odorant	ODT	ORT	ODT	ORT	ODT	ORT
2-methoxy-5-vinylphenol[a]	0.35	0.15	5.2	1.2	5.8	2.2
2-methoxy-4-vinylphenol	19[b]	5.1[b]	50[c]	nd[d]	80[e]	nd

[a] Odor thresholds were determined in this study. [b] Czerny et al. (in preparation). [c] Odor threshold taken from ref *35*. [d] Not determined. [e] Odor threshold taken from ref *4*.

retention indices (cf. **Table 2**). Because, except for the determination of an odor threshold in water (*32*), no systematic study on the aroma properties of 2-methoxy-5-vinylphenol has earlier been published, its odor quality and odor thresholds were determined. The data revealed a very low odor threshold of **18** in water as well as in oil or cellulose, which were by factors of 55, 10, or 13 lower than those of 2-methoxy-4-vinylphenol (**Table 3**).

Besides the two phenols, also β-damascenone and the methyl esters of 2- and 3-methylbutanoic acid were strongly increased in the stored sample (**Table 2**). On the other hand, in particular (*E*,*E*)-2,4-nonadienal was lower in A, 20%−40 °C, as compared to the unstored beans (REF).

Quantitation of Key Odorants. *Comparison of Unstored (REF) and Stored Beans (Sample A, 20%−40 °C).* To confirm the data obtained by application of the AEDA on the unstored and stored coffee beans, five odorants showing the largest differences in their FD factors between both samples, namely, 2-methoxy-4-vinylphenol, 2-methoxy-5-vinylphenol, methyl 2-methylbutanoate, methyl 3-methylbutanoate, and (*E*)-β-damascenone, were quantified by SIDA. In addition, (*E*,*E*)-2,4-nonadienal and 2-methoxy-3-isopropylpyrazine as well as (*E*)-2-nonenal, previously suggested as off-odorant (*12*), were also quantified.

In agreement with the results of the AEDA, the data showed (**Table 4**) that 2-methoxy-4-vinylphenol increased by a factor

Table 4. Concentrations of Selected Odorants in Unstored Coffee Beans (REF) and Raw Coffee Beans Stored for 9 Months at 40 °C in a 20% Oxygen Atmosphere (A, 20%–40 °C)

	concn[a] (μg/kg)	
odorant	REF	A
2-methoxy-4-vinylphenol	390	15000
2-methoxy-5-vinylphenol	<1	250
methyl 2-methylbutanoate[b]	9	500
methyl 3-methylbutanoate[b]	0.6	80
(*E*)-β-damascenone	0.8	29
(*E*)-2-nonenal	9	15
(*E*,*E*)-2,4-nonadienal	3.7	0.4
2-methoxy-3-isopropylpyrazine	1.7	1.6

[a] Mean values based on the analysis of triplicates. SD ≤ 10%. [b] Isomers were separately quantified in the EI mode using *m*/*z* 88 (methyl 2–methylbutanoate) and *m*/*z* 74 (methyl 3-methylbutanoate) for differentiation.

of nearly 26 from 390 to 15000 μg/kg and 2-methoxy-5-vinylphenol, which was not detectable in REF, reached a concentration of 250 μg/kg after storage. Also, the two methyl esters and (*E*)-β-damascenone showed a very strong increase in their concentrations during the 9 months of storage. On the other hand, (*E*)-2-nonenal did not much increase, whereas 2-methoxy-3-isopropylpyrazine remained constant and (*E*,*E*)-2,4-nonadienal decreased significantly. The much higher amounts of both smoky-smelling phenols were well in line with the more intense smoky aroma note in the stored sample (cf. **Figure 2**). Contrarily, the decrease in the earthy note was not reflected in the decrease of, for example, 2-methoxy-3-isopropylpyrazine. Obviously this odor quality is masked in the overall aroma by the very high odor activity of the two phenols showing odor activity values of 65 or 185, respectively, on the basis of their odor threshold in cellulose and their concentration of 390 or 15000 μg/kg.

Although there are distinct indications in the literature (*12*, *36*) that the precursors of the woody off-flavor of the beverage are derived from components of the lipid fraction, the results of the AEDA and the quantitation showed that aroma active degradation products of unsaturated fatty acids were not formed during storage. For example, the concentration of (*E*,*E*)-2,4-nonadienal, which is formed in an autoxidation of linoleic acid (*37*), was even lower in A, 20%−40 °C, and the concentration of (*E*)-2-nonenal was hardly doubled during storage (**Table 4**). Therefore, these data indicate that raw coffee beans are not a potent source of (*E*)-2-nonenal. If present in the beverage, the lipid peroxidation should take place during roasting.

Influence of Storage Time, Temperature, and Moisture and Oxygen Contents. During storage of raw coffee beans, several parameters may influence the generation of the aroma compounds under investigation. Thus, in a first series of additional experiments, the time course of odorant formation in stored coffee beans with 13.5% water content (A, 20%−40 °C) was followed. The results showed (**Table 5**) that even after a short storage period of 3 months, 2-methoxy-4-vinylphenol, all four esters, and (*E*)-β-damascenone were significantly increased. However, whereas 2-methoxy-4-vinylphenol reached a maximum after 9 months of storage, the two methyl esters continuously increased with time, reaching concentrations in the milligrams per kilogram range after 12 months of storage. Also, (*E*)-β-damascenone increased with time, but did not show a maximum within the 12 month storage period. Interestingly, 2-methoxy-5-vinylphenol was not detectable at shorter storage times, but increased considerably after 9 or 12 months, respectively. Both (*E*)-2-nonenal and (*E*,*E*)-2,4-nonadienal

Table 5. Influence of Storage Time on the Concentrations of Key Odorants in Raw Coffee Beans (A, 20%–40 °C)

odorant	concn[a] (μg/kg)					
	REF	3[b]	7[b]	9[b]	10[b]	12[b]
2-methoxy-4-vinylphenol	390	3250	7500	15000	13700	11000
2-methoxy-5-vinylphenol	<1	<1	<1	250	nd[d]	1970
methyl 3-methylbutanoate[c]	9	340	1100	500	1680	18300
methyl 2-methylbutanoate[c]	0.6	45	180	80	290	3120
(*E*)-β-damascenone	0.8	5.9	21	29	42	55
(*E*)-2-nonenal	9	13	30	15	20	10
(*E*,*E*)-2,4-nonadienal	3.7	5	0.7	0.4	0.7	1
ethyl 3-methylbutanoate	49	170	120	nd	150	nd
ethyl 2-methylbutanoate	7.3	39	29	nd	48	nd

[a] Mean values based on three to six determinations differed by no more than ≤10%. [b] Storage time in months. [c] Isomers were separately quantified in the EI mode using *m*/*z* 88 (methyl 2–methylbutanoate) and *m*/*z* 74 (methyl 3-methylbutanoate) for differentiation. [d] Not determined.

Table 6. Time Course of the Formation of Odorants in Raw Coffee Beans with a Water Content of 11.8% Stored at 25 °C under a 20% Oxygen Atmosphere (B, 20%–25 °C)

odorant	concn[a] (μg/kg)					
	REF	4[b]	7[b]	10[b]	12[b]	18[b]
2-methoxy-4-vinylphenol	230	340	430	440	690	1200
2-methoxy-5-vinylphenol	<1	<1	<1	<1	<1	<1
methyl 2- and 3-methylbutanoate[c]	10	67	100	84	150	350
(*E*)-β-damascenone	0.8	0.7	1	0.8	1.4	1.9
(*E*)-2-nonenal	19	20	14	16	19	20
(*E*,*E*)-2,4-nonadienal	7	7	6	12	7	6
ethyl 3-methylbutanoate	32	46	59	46	69	48
ethyl 2-methylbutanoate	8	10	15	13	19	11

[a] Single determinations. [b] Storage time in months. [c] Quantified as sum.

decreased after going through a transient maximum after 7 or 3 months, respectively. To elucidate their role for the fruity odor quality of the stored coffee, the ethyl esters of 2- and 3-methylbutanoic acid were additionally quantified in the six samples (**Table 5**). The data revealed that these esters were slightly increased after 3 months, but did not show a clear trend with further storage. On the basis of literature results (*6*), it can be speculated that the ethyl esters are formed by microorganisms in the samples, whereas the methyl esters are probably formed by the action of endogenous coffee enzymes.

In the following series of experiments the same batch of coffee beans was stored at 25 °C and a water content of 11.75% under an atmosphere of 20% oxygen (B, 20%−25 °C). These conditions are similar to storage parameters in regions with moderate climate. The reduction in temperature and water content as compared to the A, 20%−40 °C, sample clearly affected the generation of the aroma compounds during storage (**Table 6**). For example, even after 18 months 2-methoxy-4-vinylphenol did not reach the concentrations found after 3 months at the higher temperature and water content, respectively (**Tables 5** and **6**). 2-Methoxy-5-vinylphenol was not even formed after an extended storage time of 18 months. However, although the increase in concentration was also lowered, methyl 2- and 3-methylbutanoate were continuously formed during storage, whereas, again, the concentrations of the ethyl esters did not show a clear trend. The generation of (*E*)-β-damascenone, however, could be avoided under these conditions (**Table 6**).

In the last series of experiments, the storage time was held constant for 9 months, but the water content, the oxygen content, and the temperature were varied (**Table 7**). The results obtained for a constant storage time of 9 months are contrasted to the data measured for the unstored raw coffee beans (REF) and the beans with a water content of 13.5% stored at 40 °C and a 20% oxygen atmosphere (A, 20%−40 °C).

From a comparison of samples A, 20%−40 °C, and A, 20%−12 °C (**Table 7**), the influence of the storage temperature becomes obvious. The data indicate that lowering the temperature from 40 to 12 °C completely avoided the formation of 2-methoxy-5-vinylphenol and β-damascenone. As compared to the reference, only the esters as well as (*E*)-2-nonenal increased slightly in the sample stored at 12 °C (REF and A, 20%−12 °C, in **Table 7**).

Lowering the water content from 13.5 to 6.2% (cf. samples A, 20%−40 °C, and C, 20%−40 °C) showed the same effect for both vinylphenols and (*E*)-β-damascenone as found for the lower temperatures (**Table 7**). However, the reduced water content inhibited not only the formation of both vinylphenols and β-damascenone but also the formation of the methyl and ethyl esters. Therefore, although the temperature was high (40 °C) during the storage of sample C, 20%−40 °C, the water content turned out to be the most critical parameter in raw coffee storage. Contrarily, lowering the oxygen content, which might reduce the generation of lipid oxidation products such as (*E*)-2-nonenal and (*E*,*E*)-2,4-nonadienal, did not show a clear influence on their concentrations (**Table 7**; cf. A, 20%−40 °C, with A, 2%−40 °C).

The data suggest in particular the previously unknown coffee constituent 2-methoxy-5-vinylphenol as an indicator for an inappropriate storage of raw coffee beans, because this odorant was detected only during storage at higher temperature and a higher water content in the beans. It can be assumed that this compound is formed by a decarboxylation of isoferulic acid as previously shown for the formation of 2-methoxy-4-vinylphenol from thermally degraded ferulic acid (*38*). However, in this study a higher temperature (40 °C) alone did not lead to the formation

Table 7. Influence of Moisture, Temperature, and Oxygen Content on the Generation of Aroma Compounds during Storage of Raw Coffee Beans at 9 Months

odorant	concn[a] (μg/kg) in sample				
	REF	A, 20%–40 °C	A, 2%–40 °C	A, 20%–12 °C	C, 20%–40 °C
2-methoxy-4-vinylphenol	390	15000	12600	290	340
2-methoxy-5-vinylphenol	<1	250	<1	<1	<1
methyl-2- and 3-methylbutanoate[b]	9.6	580	1570	48	11
(*E*,*E*)-2,4-nonadienal	3.7	0.4	1	5	8
(*E*)-β-damascenone	0.8	29	25	<1	0.7
(*E*)-2-nonenal	9	15	32	23	22
ethyl 2-methylbutanoate	7.3	nd[c]	110	18	5
ethyl 3-methylbutanoate	49	nd	420	110	18

[a] Mean values based on three to six determinations differed by no more than ≤15%. [b] Quantified as sum. [c] Not determined.

of 2-methoxy-4-vinylphenol, but at a higher water content (13.5%) it was formed in considerable amounts. This may indicate an enzymatic rather than a thermally induced decarboxylation, because at a lower water content (C samples) and higher temperature (40 °C), 2-methoxy-4-vinylphenol was not generated. In preliminary studies (data not shown), 2-methoxy-5-vinylphenol was also detected in 7-year-old raw Arabica coffee beans from Colombia in concentrations of 1340 μg/kg, so this odorant may be a useful storage indicator for raw coffee beans.

Roberts and Acree (*39*) suggested the acetylenic diol 3-hydroxy-7,8-dihydro-β-ionol as precursor of (*E*)-β-damascenone, which, when heated in an acidic environment, yields 3-hydroxy-β-damascenone as the main product and (*E*)-β-damascenone as a minor product. Glycosides of the acetylenic diol, for example, the C9-β-D-glucopyranoside (*40*), may also liberate (*E*)-β-damascenone when heated at low pH. Our results reveal that the generation of (*E*)-β-damascenone during the storage of raw coffee needs a higher temperature together with a higher water content. It can, therefore, be assumed that the same precursors are present in raw coffee beans as reported in wine (*40*), but the hydrolysis of the glycoside may be caused by enzymes of the coffee or the microorganisms.

In their investigations on the overfermented flavor defect of coffee, Bade-Wegner et al. (*6*) determined the generation of ethyl 2- and 3-methylbutanoate in green coffee during storage at 30 °C as a function of moisture level and time. They observed that with increasing moisture level and storage time, the contents of the esters went up dramatically, whereas the contents in a short-time steam-treated coffee remained unaffected even at moisture levels of 20%. The authors, thus, came to the conclusion that elevated moisture levels and temperatures support growth of microorganisms, which generate the esters during shipping or storage.

In our experiments an increase of the concentrations of the ethyl, but moreover of the methyl, esters of 2- and 3-methylbutanoic acid was also forced by a higher water content and temperature. Therefore, a generation of the esters by microorganisms must be considered as a possible pathway, but forced ester generation may also be induced by reactions of coffee enzymes.

In summary, the results indicate that a few months of storage of raw coffee in a damp and warm climate may cause significant changes in the concentrations of important key odorants of raw coffee. Whether these changes during storage of raw coffee have an influence on the quality of the roasted coffee and its beverage is the subject of further investigations.

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