

Changes in Key Aroma Compounds of Criollo Cocoa Beans During Roasting

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Application of a comparative aroma extraction dilution analysis on unroasted and roasted Criollo cocoa beans revealed 42 aroma compounds in the flavor dilution (FD) factor range of 1–4096 for the unroasted and 4–8192 for the roasted cocoa beans. While the same compounds were present in the unroasted and roasted cocoa beans, respectively, these clearly differed in their intensity. For example, 2- and 3-methylbutanoic acid (rancid) and acetic acid (sour) showed the highest FD factors in the unroasted beans, while 3-methylbutanal (malty), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (caramel-like), and 2- and 3-methylbutanoic acid (sweaty) were detected with the highest FD factors in the roasted seeds. Quantitation of 30 odorants by means of stable isotope dilution assays followed by a calculation of odor activity values (ratio of the concentration/odor threshold) revealed concentrations above the odor threshold for 22 compounds in the unroasted and 27 compounds in the roasted cocoa beans, respectively. In particular, a strong increase in the concentrations of the Strecker aldehydes 3-methylbutanal and phenylacetaldehyde as well as 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was measured, suggesting that these odorants should contribute most to the changes in the overall aroma after roasting. Various compounds contributing to the aroma of roasted cocoa beans, such as 3-methylbutanoic acid, ethyl 2-methylbutanoate, and 2-phenylethanol, were already present in unroasted, fermented cocoa beans and were not increased during roasting.

KEYWORDS: Cocoa beans; roasting process; aroma extraction dilution analysis; stable isotope dilution assay

INTRODUCTION

The attractive aroma of roasted cocoa is the result of a sophisticated technological process applied to the seeds of the cocoa tree (cocoa beans, *Theobroma cacao* L.). Among the manufacturing steps, both, fermentation and roasting are considered to be the most important with respect to flavor formation, because, e.g., roasting of nonfermented cocoa does not deliver the characteristic cocoa aroma (1). During fermentation, aroma precursors, such as free amino acids, short-chain peptides, and reducing sugars, are formed (2–7), from which the typical cocoa aroma is suggested to be generated during the subsequent roasting process. Besides, also a significant increase in volatile compounds, such as alcohols, organic acids, and aldehydes, was found after fermentation (8). In particular, 2-phenylethanol is formed in significant amounts, a compound which has later been reported as an important aroma compound in cocoa mass (9). Moreover, also an increase in the concentration of 3-methylbutanal has been observed (10, 11), a compound quite often proposed as an important aroma compound in roasted cocoa (3, 12, 13).

Roasting of the fermented seeds fulfills mainly two purposes: the removal of undesired compounds with low boiling points, such as acetic acid, and the formation of the typical roasty, sweet odorants of cocoa (14). The thermal treatment initiates reactions between reducing sugars and free amino acids or short-chain peptides, consequently leading to a significant reduction in the concentration of free amino acids and reducing sugars (17–19). Ziegler and Biehl (18) observed that the formation of 3-methylbutanal during roasting depends upon the quality of the preceding fermentation procedure. While the concentration of 3-methylbutanal was only 4–10 mg/kg in poorly fermented beans, well-fermented seeds delivered 60 mg/kg after roasting. Further compounds formed during roasting are alcohols, ethers, furans, thiazoles, pyrones, acids, esters, imines, amines, oxazoles, and pyrroles (19, 20).

However, although numerous investigations on the aroma of cocoa have been published thus far (21), only a very few authors have used a comprehensive combination of analytical and sensory methods to differentiate key odorants from the numerous non-odor active volatiles. Schnermann and Schieberle (9) were the first to apply the aroma extract dilution analysis (AEDA) on a commercial cocoa mass. Their results revealed, in particular, 3-methylbutanal, ethyl 2-methylbutanoate, hexanal, 2-isopropyl-3-methoxypyrazine, 2-ethyl-3,5-dimethylpyrazine,

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Table 1. Selected Ions and Response Factors Used in the Stable Isotope Dilution Assays

odorant ^a	ion (<i>m/z</i>)	internal standard	number	ion (<i>m/z</i>)	RF ^b
2-methylbutanal ^c	69	[² H ₂]-2-methylbutanal	d-2a	70 + 71	0.78
3-methylbutanal ^c	69	[² H ₂]-3-methylbutanal	d-2b	71	0.69
ethyl-2-methylpropanoate	117	[² H ₃]-ethyl-2-methylpropanoate	d-3	120	0.92
ethyl-2-methylbutanoate	137	[² H ₃]-ethyl-2-methylbutanoate	d-4	140	0.95
1-octen-3-one	127	[² H ₂]-1-octen-3-one	d-8	129	0.90
2-heptanol	99	[² H ₄]-2-heptanol	d-9	103	0.79
2-acetyl-1-pyrroline ^c	112	[² H ₂₋₅]-2-acetyl-1-pyrroline	d-10	114 + 117	0.83
dimethyl trisulfide	127	[² H ₆]-dimethyl trisulfide	d-11	133	1.0
2,3,5-trimethylpyrazine	123	[² H ₂]-2,3,5-trimethylpyrazine	d-13	126	0.93
2-ethyl-3,5-dimethylpyrazine	137	[² H ₃]-2-ethyl-3,5-dimethylpyrazine	d-15	140	0.93
2-ethyl-3,5-dimethylpyrazine	137	[² H ₃]-2-ethyl-3,5-dimethylpyrazine	d-15	140	0.95
2,3-diethyl-5-methylpyrazine ^c	151	[² H ₃]-2,3-diethyl-5-methylpyrazine	d-17	154	0.95
acetic acid ^d	61	[² H ₃]-acetic acid	d-18	64	0.87
3-isobutyl-2-methoxy-pyrazine	167	[² H ₃]-3-isobutyl-2-methoxy-pyrazine	d-19	170	0.90
methylpropanoic acid ^d	89	[² H ₂]-methylpropanoic acid	d-22	91	1.0
butanoic acid ^d	103	[² H ₂]-butanoic acid	d-23	105	0.85
2-methyl-3-(methylthio)furan ^c	161	[² H ₃]-2-methyl-3-(methylthio)furan	d-25	164	0.90
(<i>E,E</i>)-2,4-nonadienal	139	[² H ₂]-(<i>E,E</i>)-2,4-nonadienal	d-26	141	0.87
2/3-methylbutanoic acid ^d	103	[² H ₂]-3-methylbutanoic acid	d-27	105	0.67
2-methoxyphenol ^d	125	[² H ₃]-2-methoxyphenol	d-29	128	0.98
2-phenylethanol ^d	105	[² H ₂]-2-phenylethanol	d-30	107	0.94
δ-octenolactone	141	[² H ₂]-δ-octenolactone	d-32	145	0.98
4-methylphenol ^c	109	[² H ₂]-4-methylphenol	d-35	115 + 116	1.0
δ-decenolactone	169	[² H ₂]-δ-decenolactone	d-38	171	0.91
linalool ^c	137	[¹³ C ₂]-linalool	c-21	139	1.0
phenylacetaldehyde	121	[¹³ C ₂]-phenylacetaldehyde	c-24	123	1.0
2-phenylethyl acetate ^d	166	[¹³ C ₂]-2-phenylethyl acetate	c-28	168	1.0
2-phenylethanol ^d	105	[¹³ C ₂]-2-phenylethanol	c-30	107	1.0
4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone ^{c,d}	129	[¹³ C ₂]-4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone	c-34	131	1.0
3-hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone ^{c,d}	129	[¹³ C ₂]-3-hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone	c-39	131	1.0
phenylacetic acid ^d	137	[¹³ C ₂]-phenylacetic acid	c-42	139	1.0

^a Compounds were quantified after separation on an OV-1701 GC stationary phase by mass spectrometry in the MS-Cl mode. ^b The response factor (RF) was determined as reported previously (37). ^c The quantitation was performed by means of two-dimensional GC-MS. ^d The quantitation was performed on the FFAP column.

as well as 2- and 3-methylbutanoic acids as important contributors to the aroma. The important role of organic acids for the cocoa aroma was later confirmed by Krings et al. (22).

Recently, on the basis of the odor activity value concept, the key aroma compounds of a commercial cocoa powder were identified (23). Among the compounds identified and quantified, 24 odorants were present in concentrations above their odor thresholds, among which acetic acid, 2- and 3-methylbutanal, 3-methylbutanoic acid, and phenylacetaldehyde showed the highest odor activity values (ratio of concentration/odor threshold). Finally, reconstitution experiments, in which the 24 odorants were combined in the same concentration as they occurred in the cocoa powder, revealed that the aroma of the cocoa powder could be successfully mimicked.

Despite the fact that roasting is among the most important steps in generating the key odorants of cocoa, comprehensive studies aimed at clarifying changes in the key aroma compounds between fermented, unroasted and fermented, roasted beans performed on the same batch are scarcely available. Thus, the aim of this work was to compare the key odorants present in raw fermented cocoa beans to those formed after roasting. The results should give insights into which odorants are delivered by the fermented, raw bean itself and which are formed from odorless during roasting.

MATERIALS AND METHODS

Cocoa Beans. Fermented and dried Criollo cocoa beans (*Theobroma cacao* L.) from Grenada were supplied by a chocolate producing company. Roasting of the beans was performed in a laboratory scale with a coffee-roaster (Probat BRZ 4, Emmerich, Germany). Cocoa beans were freshly roasted for each experiment, and roasting conditions were optimized by variation of roasting time and temperature. The overall aroma generated was checked by sensorial evaluation of the roasted samples in comparison to

industrially roasted samples. A temperature of 95 °C applied for 14 min was finally judged to deliver the characteristic and desired aroma of roasted cocoa beans.

Reference Odorants. The following reference compounds were obtained from the sources given in parentheses: *p*-cresol, (*R/S*)- γ -decalactone, (*R/S*)- δ -decalactone, 2,3-diethyl-5-methylpyrazine, dimethyl trisulfide, 2-ethyl-3,5-dimethylpyrazine, (*R/S*)-ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 2-heptanol, 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone, 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, *cis*-isoeugenol, (*R/S*)-linalool, 2-methoxy-3-isobutylpyrazine, (*R/S*)-2-methylbutanal, 3-methyl-1-butanol, (*R/S*)-2-methylbutanoic acid, 3-methylbutanoic acid, 3-methylindole, methylpropanoic acid, (*E,E*)-2,4-nonadienal, (*R/S*)- γ -nonalactone, 1-octen-3-one, phenylacetaldehyde, phenylacetic acid, 2-phenylethanol, 2-phenylethyl acetate, propanoic acid, benzyl alcohol, and 2,3,5-trimethylpyrazine (Sigma-Aldrich Chemie, Taufkirchen, Germany); butanoic acid, acetic acid, and ethyl 2-methylpropanoate (Merck, Darmstadt, Germany); 2-methoxyphenol (Serva, Heidelberg, Germany); and 3-methylbutanal (Lancaster, Mülheim, Germany). The following odorants were synthesized as previously reported (23): 2-acetyl-1-pyrroline, 2-methyl-3-(methylthio)furan, and δ -decenolactone.

Isotopically Labeled Internal Standards. The isotopically labeled internal standards, either labeled with deuterium or carbon-13, were synthesized as described in the references given in parentheses. Numbering refers to the numbers of the respective odorants described in Table 1 but assigned as "d" for deuterated isotopologues and "c" for carbon-13-labeled ones: d-2a (24); d-2b (25); d-3 and d-4 (26); d-5 and d-10 (27); d-11 (28); d-13 and c-28 (Czerny, unpublished); d-15, d-17, and d-29 (29); d-19 (30); c-21 (31); d-22 (32); d-23 (33); d-25 and d-38 (24); d-27 (34); d-30 and c-24 (35); d-32 (36); c-34 (37); c-39 (38). [²H₃]-Acetic acid (d-18), [²H₃]-2-methoxyphenol (d-29), and [¹³C₂]-phenylacetic acid (c-42) were purchased from Sigma-Aldrich Chemie, Taufkirchen, Germany.

Syntheses. [²H₄]-2-Heptanol (d-9). In a laboratory autoclave equipped with a glass vial (100 mL), tris-(triphenylphosphine)-rhodium(I)-chloride (Wilkinson catalyst, 0.8 g) was suspended in toluene (15 mL) under a nitrogen atmosphere. Then, nitrogen was replaced by deuterium,

and the mixture was stirred until the dark-red-colored suspension turned into orange. 4-Heptene-2-ol (2.5 g) was added; the mixture was stirred for 8 h; and finally, pentane (50 mL) was added. The catalyst was removed by high vacuum distillation, and the distillate was concentrated to 15 mL by distilling off the solvent using a Vigreux column (60 × 1 cm). To remove the toluene, the distillate was applied onto a water-cooled column (30 × 1.5 cm) filled with silica gel (30 g). The column was first flushed with pentane (250 mL), and then the target compound was eluted with diethyl ether (150 mL). The eluate was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The incorporation of the deuterium atoms was confirmed by mass spectrometry (MS–CI): m/z in %, 103 (100), 102 (77), 60 (50), 101 (36), 100 (20), 65 (14). The main fragment m/z 103 is formed by elimination of water ($M^+ - 18 + 1 = 103$) from the molecular ion of [$^2\text{H}_4$]-2-heptenol m/z 120.

[$^2\text{H}_2$]-(*E,E*)-2,4-Nonadienal (*d*-26). [$^2\text{H}_2$]-(*E,E*)-2,4-nonadienal was synthesized via a Grignard reaction using 1-methoxybut-1-en-3-ine and [$^2\text{H}_2$]-pentanal following a procedure described for [$^2\text{H}_2$]-(*E,E*)-2,4-decadienal (26). 1-Methoxybut-1-en-3-ine was synthesized according to Shostakovskii and Khomenko (39). [$^2\text{H}_2$]-Pentanal was synthesized as described above for [$^2\text{H}_4$]-2-heptanol but using 2-pentenal instead of 4-hepten-2-ol in the deuteration process. The incorporation of the two deuterium atoms was checked by MS–CI: m/z in %, 141 (100), 142 (18), 140 (13), 123 (8). The molecular ion m/z 141 ($M^+ + 1$) shows a shift of two mass units as compared to the unlabeled dienal (m/z 139), thus confirming the success of the labeling procedure.

Isolation of the Volatile Fraction. For solvent extraction, cocoa beans were frozen with liquid nitrogen and then ground into a fine powder in a laboratory mill (Model A 10, Jahnke and Kunkel, Staufen, Germany). The material (50 g) was extracted with diethyl ether (2 × 300 mL) by vigorous stirring at room temperature for 1 h. The extract was filtered and concentrated to about 150 mL on a Vigreux column (60 × 1 cm), and the volatiles were isolated using the solvent-assisted flavor evaporation (SAFE) method (40). Separation into acidic (AF) and neutral-basic volatiles (NBV) and concentration of the two fractions to 500 μL each were performed as previously described (23).

Gas Chromatography–Olfactometry (GC–O); Aroma Extract Dilution Analysis (AEDA). GC–O was performed by means of a Fisons Instruments type 8160 gas chromatograph (Mainz, Germany) using the fused silica capillaries and the temperature programs described recently (24). Samples were applied by the cold on-column injection technique at 40 °C with helium as the carrier gas at a flow rate of 2.2 mL/min. For GC–O and AEDA, the effluent was split 1:1 (by volume) at the end of the column via two deactivated fused silica capillaries (40 cm × 0.15 mm i.d.). One part was directed to a flame ionization detector (FID) held at 200 °C, and the other one was directed to a sniffing port held at 180 °C. Both the neutral-basic and the acidic fraction were stepwise-diluted with diethyl ether (1:1, v/v) and analyzed by HRGC–O until no odor-active area was detectable. Odorants were assigned a FD factor ≥ 1 (41). HRGC–O was performed by injecting aliquots of 0.5 μL , and linear retention indices (RIs) of the compounds were calculated using a series of *n*-alkanes (23).

High-Resolution Gas Chromatography–Mass Spectrometry (HRGC–MS). For compound identification, mass spectra of the analyte and the reference compound were measured by means of a Saturn 2000 mass spectrometer (Varian, Darmstadt, Germany) in tandem with the capillaries described above. In particular, care was taken that the reference compound elicited the same odor quality and the same odor intensity at the sniffing port to avoid wrong identifications because of coeluting compounds (41). Mass spectra were generated in the electron impact mode (MS–EI) at 70 eV and in the chemical ionization mode (MS–CI) at 115 eV using methanol as the reactant gas.

Quantitation by Stable Isotope Dilution Assays. Dependent upon the amount of the respective analyte present, which was estimated in a preliminary experiment, aliquots of ground cocoa beans (5–250 g, respectively) were suspended in diethyl ether and defined amounts of the respective labeled isotopologues (Table 1), dissolved in diethyl ether, were added. The suspension was stirred for 1 h, and then the volatile fraction was isolated as described above. The distillate was separated into the neutral-basic and the acidic fraction by treatment with aqueous sodium bicarbonate, and both fractions were analyzed

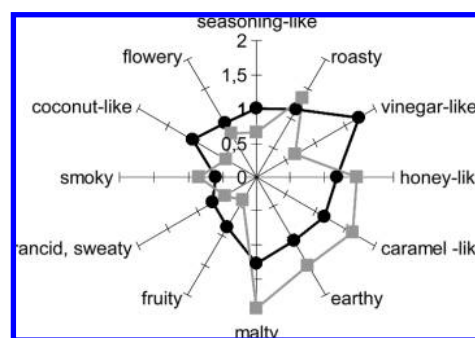


Figure 1. Orthonasal aroma profiles of unroasted (—●—) and roasted (—■—) Criollo cocoa beans.

by HRGC–MS, monitoring the intensities of the respective ions given in Table 1. To obtain unequivocal mass spectra, in some cases, two-dimensional (2D) HRGC–MS was performed using the moving column stream switching system (MCSS) (Fisons Instruments, Mainz, Germany), as recently described (42). The concentrations were calculated from the relative abundances of the ions selected for the analyte and the internal standards, and the data were corrected by means of response factors, determined from mixtures containing known amounts of the respective labeled and unlabeled compound (37).

Sensory Evaluation. Sensory analyses were performed at 21 ± 1 °C in a sensory panel room equipped with single booths. A total of 10 assessors with experience in sensory evaluation were recruited from the German Research Center of Food Chemistry in Garching, Germany. Aroma profile analyses were performed by orthonasally scoring 12 odor qualities on a 7 point scale from 0 to 3 (from 0, 0.5, 1, ..., to 3) selected in a previous session for the evaluation of unroasted and roasted Criollo cocoa beans. The values given by the panelists were averaged. Orthonasal odor thresholds were determined in sunflower oil as described recently (23).

RESULTS AND DISCUSSION

Aroma Profile Analysis. An orthonasal evaluation of the main odor qualities of the fermented, unroasted Criollo cocoa beans revealed the odor quality “vinegar-like” with the highest intensity, followed by malty, roasty, caramel-like, honey-like, coconut-like, flowery, seasoning-like, and earthy, which all were perceived at nearly the same intensity (Figure 1). Fruity, rancid-sweaty, and smoky aroma notes were scored somewhat lower. After the seeds were roasted, the aroma profile was substantially changed. The strong vinegar-like note predominating in unroasted cocoa was scored significantly lower, while the malty note was predominating, followed by caramel-like, earthy, honey-like, and roasty aroma qualities (Figure 1).

Identification of Odor-Active Compounds in Raw, Fermented Cocoa Beans. The volatile fraction from fermented, raw cocoa beans (50 g) was isolated by solvent extraction followed by high vacuum distillation. This distillate fully represented the typical aroma of the raw cocoa beans when sniffed on a strip of filter paper. Application of HRGC–O on the distillate revealed 34 aroma-active regions in the neutral-basic volatile fraction (NBV). Eight further odorants were detected in the fraction containing the acidic compounds (AF). By sniffing of serial dilutions, compound 27, exhibiting a rancid aroma, was detected with the highest flavor dilution (FD) factor of 4096, followed by 18 (sour) with a FD factor of 2048. Further important odorants showing somewhat lower FD factors were 31 (flowery), 39 (seasoning-like), and 29 (smoky) (Figure 2).

For the identification experiments, an aroma distillate was prepared from 500 g of ground cocoa beans and the neutral/basic fraction was fractionated on silica using solvent mixtures of different polarity (9). After GC–O and mass spectrometric analysis, structures of odor-active compounds were suggested

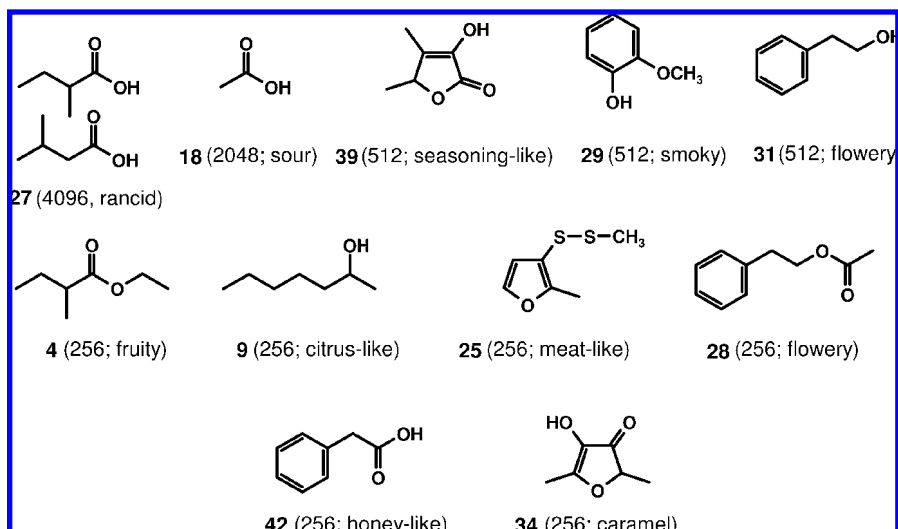


Figure 2. Structures of the most odor-active compounds in fermented unroasted cocoa.

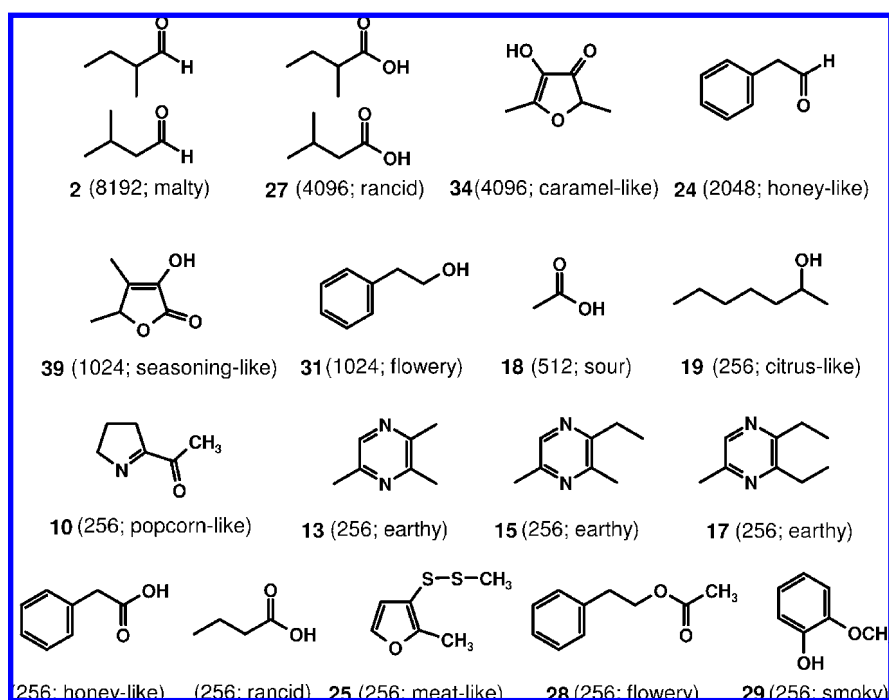


Figure 3. Structures of the most odor-active compounds in fermented, roasted Criollo cocoa beans.

on the basis of data available in an in-house database. Correct identifications of compounds were finally performed by comparison of the retention indices on two columns of different polarities, their odor qualities, and odor activities, as well as their mass spectra (MS–EI and MS–CI), to reference compounds (41). Thus, the most odor-active compounds among the neutral/basic odorants were identified as 2-phenylethanol (**31**, flowery) and 2-methoxyphenol (**29**, smoky), followed by ethyl 2-methylbutanoate (**4**, fruity), 2-heptanol (**9**, citrus-like), 2-methyl-3-(methylthio)furan (**25**, cooked meat-like), and 2-phenylethyl acetate (**28**, flowery), which were all detected with a FD factor of 256 (Figure 2). In the acid fraction, compound **27**, showing a rancid odor quality, was identified as a mixture of 2- and 3-methylbutanoic acid, compound **18**, with a sour odor as acetic acid, and compound **39**, eliciting a seasoning-like note as 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon). In addition, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (**34**, caramel-like) and phenylacetic acid (**42**, sweet) were characterized as further important odorants.

Identification of Odor-Active Compounds in Roasted Cocoa Beans. The aroma extract dilution analysis applied on a distillate from the same batch of cocoa beans, which were roasted for 14 min, revealed 42 odor-active areas. On the basis of the identification experiments in correlation with the FD factors, 2- and 3-methylbutanal (**2**), both exhibiting a malty aroma note, followed by phenylacetaldehyde (**24**, honey-like) and 2-phenylethanol (**31**, flowery) were identified with the highest FD factors between 1024 and 8192, respectively (Figure 3). A somewhat lower FD factor of 256 was found for 2-heptanol (**9**, citrus-like), 2-acetyl-1-pyrroline (**10**, popcorn-like), 2,3,5-trimethylpyrazine (**13**, earthy), 2-ethyl-3,6-dimethylpyrazine (**15**, earthy), 2,3-diethyl-5-methylpyrazine (**17**, earthy), 2-methyl-3-(methylthio)furan (**25**, cooked meat-like), 2-phenylethyl acetate (**28**, flowery), and 2-methoxyphenol (**29**, smoky). In the acidic fraction, 2- and 3-methylbutanoic acid (**27**, rancid) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (**34**, caramel-like) appeared with the highest FD factor of 4096, followed by 3-hydroxy-4,5-dimethyl-2(5H)-furanone (**39**, sea-

Table 2. Most Odor-Active Compounds Identified in the Volatile Fractions Isolated from Unroasted and Roasted Criollo Cocoa Beans

number	odorant ^a	odor quality ^b	RI ^c on			FD factor ^d	
			FFAP	SE 54	OV 1701	unroasted	roasted
1	2-methylpropanal	malty	830	—	—	1	8
2	2- ^e and 3-methylbutanal	malty	900	651	731	128	8192
3	ethyl 2-methylpropanoate	fruity	937	—	813	128	64
4	ethyl 2-methylbutanoate ^e	fruity	1031	851	906	256	64
5	ethyl 3-methylbutanoate	fruity	1047	855	908	16	16
6	unknown	fruity	1162	—	—	8	8
7	3-methyl-1-butanol	malty	1185	736	—	16	8
8	1-octen-3-one	mushroom-like	1289	981	—	4	8
9	2-heptanol	citrusy	1300	952	980	256	256
10	2-acetyl-1-pyrroline	popcorn-like	1314	927	1019	32	256
11	dimethyl trisulfide	sulfurous	1332	961	1043	64	128
12	unknown	rubber-like	1380	—	—	8	8
13	2,3,5-trimethylpyrazine	earthy	1383	1011	1081	64	256
14	unknown	potato-like	1398	—	—	4	32
15	2-ethyl-3,6-dimethylpyrazine	earthy	1421	1080	1147	4	64
16	2-ethyl-3,5-dimethylpyrazine	earthy	1430	1084	1150	64	256
17	2,3-diethyl-5-methylpyrazine	earthy	1454	1090	1152	4	256
18	acetic acid ^f	sour	1462	—	—	2048	512
19	3-isobutyl-2-methoxypyrazine	bell pepper	1489	1101	1145	64	128
20	propanoic acid ^f	rancid	1518	—	—	16	32
21	linalool ^e	flowery	1533	1192	—	16	16
22	methylpropanoic acid ^f	rancid	1538	—	—	128	128
23	butanoic acid ^f	sweaty	1605	—	—	128	256
24	phenylacetaldehyde	honey-like	1609	1039	1179	32	2048
25	2-methyl-3-(methylthio)furan	meat-like	1615	—	—	256	256
26	(<i>E,E</i>)-2,4-nonadienal	fatty, oily	1635	—	1375	8	8
27	2- ^e and 3-methylbutanoic acid ^f	rancid	1646	—	—	4096	4096
28	2-phenylethyl acetate	flowery	1780	1382	1474	256	256
29	2-methoxyphenol	smoky	1837	1087	1224	512	256
30	phenylmethanol	flowery	1844	—	—	32	16
31	2-phenylethanol	flowery	1884	1112	1282	1024	1024
32	δ -octenolactone ^e	coconut-like	2005	1260	—	8	16
33	γ -nonalactone ^e	peach-like	2009	1365	1540	4	4
34	4-hydroxy-2,5-dimethyl-3(2 <i>H</i>)-furanone ^f	caramel-like	2040	—	—	256	4096
35	<i>p</i> -cresol	faecal	2063	—	1311	32	16
36	γ -decalactone ^e	peach-like	2100	1462	—	32	32
37	δ -decalactone ^e	coconut-like	2176	—	1352	8	4
38	δ -decenolactone	coconut-like	2207	1477	1724	64	128
39	3-hydroxy-4,5-dimethyl-2(5 <i>H</i>)-furanone ^f	seasoning-like	2214	—	—	512	1024
40	<i>cis</i> -isoeugenol	smoky	2287	—	1573	32	64
41	3-methylindol	faecal	2432	—	1626	2	4
42	phenylacetic acid ^f	honey-like	2519	—	—	256	256

^a The compound was identified by comparing the mass spectra (MS—EI and MS—CI), the retention indices on capillary FFAP, SE 54, and OV 1701, and the odor quality and intensity perceived during sniffing to data obtained for the reference compound. ^b Odor quality perceived at the sniffing port. ^c Retention index; —, not determined. ^d FD factor determined by AEDA on the FFAP column. ^e Stereochemistry was not determined. ^f Compound was identified in the acidic fraction.

soning-like) and acetic acid (**18**, sour). All odorants identified are summarized in **Table 2**.

On the basis of a comparison of the key odorants in both samples (**Table 2**), the following conclusions could be drawn: Only a quite limited number of important odorants in roasted cocoa, such as 2- and 3-methylbutanal (**2**), phenylacetaldehyde (**24**), 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (**34**), and 2,3-diethyl-5-methylpyrazine (**17**), were clearly formed from precursors in the fermented cocoa beans. On the other hand, many aroma compounds, such as 2- and 3-methylbutanoic acid (**27**), 2-phenylethanol (**31**), 3-hydroxy-4,5-dimethyl-2(5*H*)-furanone (**39**), 2-methyl-3-(dithio)-furan (**25**), or phenylacetic acid (**42**), were already present in considerable amounts in the raw seeds.

Quantitation of Key Odorants in Raw and Roasted Cocoa Beans. Following the protocol of an aroma extract dilution analysis, losses during the workup procedure are not taken into account. Thus, to obtain more precise data on the source of key odorants in the roasted beans, a total of 31 odorants was quantified by means of stable isotope dilution assays, which were previously developed (**23**).

The results obtained for the unroasted cocoa beans showed that acetic acid was by far the most abundant odorant (1.1 g/kg), followed by methylpropanoic acid and 3-methylbutanoic acid with amounts of 9.7 and 9.3 mg/kg, respectively (**Table 3**). Quite high concentrations were also found for 2-phenylethanol (3.5 mg/kg), 3-methylbutanal (1.6 mg/kg), and 2-heptanol (1.16 mg/kg). On the other hand, some components were only present in trace amounts, such as 1-octen-3-one (0.21 μ g/kg) and 2-methyl-3-(methylthio)-furan (0.13 μ g/kg). As expected from the AEDA results, quantitation of the odorants after roasting revealed significant differences in the concentrations of several aroma compounds. For example, phenylacetaldehyde increased by a factor of more than 90, while 3-methylbutanal increased by a factor of more than 16 in the roasted sample. In addition, the amount of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was increased more than 70 times during roasting. Interestingly, pyrazines, commonly suggested as compounds only formed during thermal food processing, were already present in the unroasted beans in significant amounts and increased only moderately during roasting. Only acetic acid was significantly lowered, while even the very volatile esters ethyl 2-methylbu-

Table 3. Concentrations ($\mu\text{g}/\text{kg}$) of 30 Odor-Active Compounds in Unroasted and Roasted Criollo Cocoa Beans

odorant	unroasted beans		roasted beans	
	mean value	SD ^a	mean value	SD ^a
acetic acid	1100000	100700	330000	8500
3-methylbutanal	1600	60	33900	610
methylpropanoic acid	9700	189	13900	410
3-methylbutanoic acid	9330	522	9700	270
2-phenylethanol	3500	4.5	7500	230
2-phenylacetic acid	5080	176	5700	570
phenylacetaldehyde	65	4.0	5500	6.5
2-methylbutanal ^b	560	30	4500	16
2-methylbutanoic acid ^b	3370	238	3500	99
4-hydroxy-2,5-dimethyl-3(2H)-furanone	17	0.35	1200	18
2-heptanol	1160	35	1070	89
2-phenylethyl acetate	940	6.8	930	6
2,3,5-trimethylpyrazine	170	8.0	920	6.5
butanoic acid	570	0.50	570	19
2-methoxyphenol	110	7.5	230	1.0
linalool ^b	120	1.0	130	0.5
δ -decalactone ^b	44	1.2	73	3.8
2-ethyl-3,6-dimethylpyrazine	19	1.3	56	4.6
dimethyl trisulfide	9.1	0.03	53	0.65
ethyl 2-methylbutanoate	36	1.7	35	0.20
ethyl methylpropanoate	31	2.1	26	0.35
δ -octenolactone ^b	17	0.40	18	2.3
2-ethyl-3,5-dimethylpyrazine	5.0	0.29	17	0.30
3-hydroxy-4,5-dimethyl-2(5H)-furanone	8.6	0.40	13	0.30
4-methylphenol	5.3	0.50	9.9	0.74
2-acetyl-1-pyrroline	nd ^c	—	4.2	0.53
2,3-diethyl-5-methylpyrazine	1.2	0.14	3.3	0.20
(E,E)-2,4-nonadienal	0.94	0.02	3.0	0.07
1-octen-3-one	0.21	0.01	1.5	0.01
2-methoxy-3-isobutylpyrazine	1.0	0.03	0.94	0.02
2-methyl-3-(methylthio)furan	0.13	0.01	0.59	0.09

^a Standard deviation (SD) calculated from quantitative data obtained from at least three different samples. ^b Stereoisomers, if present, were not separately determined. ^c nd = not determined.

tanoate and ethyl methylpropanoate remained nearly constant. The same was true for 2- and 3-methylbutanoic acid, 2-phenylethanol, 2-phenylethylacetate, or 2-heptanol, respectively (Table 3).

Calculation of Odor Activity Values (OAVs). To elucidate whether the quantified compounds were present in concentrations above their odor thresholds and may, therefore, contribute to the overall aroma of the cocoa beans, odor activity values (ratios of concentration/orthonasal odor threshold) of the aroma compounds were calculated. Because cocoa beans contain about 54% fat (43), sunflower oil was chosen as an appropriate matrix for threshold determination. The threshold values for most compounds have previously been determined by our group (23, 44), except the value for 2-heptanol, which was newly determined in this study (Table 4).

In unroasted cocoa beans, 22 aroma compounds were present in concentrations above their odor thresholds (Table 4). By far the highest OAV of 8870 was calculated for acetic acid, followed by 3-methylbutanoic acid (424), ethyl 2-methylbutanoate (138), and 3-methylbutanal (123). Methylpropanoic acid, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, ethyl 2-methylpropanoate, 2-methylbutanoic acid, 2-phenylethanol, and 2-phenylacetic acid showed OAVs > 10 and are, thus, also likely to contribute to the aroma of the unroasted cocoa beans.

In roasted cocoa beans, 27 compounds were present in concentrations exceeding their odor thresholds (Table 4). Because of the changes in concentrations of several compounds caused by the roasting process, however, the ranking of compounds on the basis of their OAVs was clearly different. Although the highest value was still calculated for acetic acid

Table 4. Odor Thresholds and OAVs of Important Aroma Compounds in Unroasted and Roasted Cocoa Beans

odorant	odor threshold ^a ($\mu\text{g}/\text{kg}$)	OAV ^b	
		unroasted beans	roasted beans
acetic acid	124 ^c	8870	2660
3-methylbutanoic acid	22 ^c	424	440
ethyl 2-methylbutanoate	0.26 ^c	138	135
3-methylbutanal	13 ^c	123	2610
methylpropanoic acid	190 ^d	51	73
3-hydroxy-4,5-dimethyl-2(5H)-furanone	0.2 ^c	43	65
ethyl 2-methylpropanoate	1.24 ^c	25	21
2-methylbutanoic acid	203 ^e	17	17
2-phenylethanol	211 ^c	17	36
2-phenylacetic acid	360 ^c	14	16
2-methoxyphenol	16 ^c	6.9	14
2-heptanol	263	4.4	4.1
butanoic acid	135 ^c	4.2	4.2
2-methylbutanal	140 ^c	4.0	32
2-phenylethyl acetate	233 ^e	4.0	4.0
dimethyl trisulfide	2.5 ^c	3.6	21
linalool	37	3.2	3.5
phenylacetaldehyde	22 ^c	3.0	250
2,3-diethyl-5-methylpyrazine	0.5 ^c	2.4	6.6
δ -octenolactone	4730 ^e	2.3	2.4
2-ethyl-3,5-dimethylpyrazine	2.2 ^c	2.3	7.6
2-isobutyl-3-methoxy-pyrazine	0.8 ^c	1.3	1.2
4-hydroxy-2,5-dimethyl-3(2H)-furanone	25 ^c	<1	48
4-methylphenol	68 ^d	<1	<1
2-ethyl-3,6-dimethylpyrazine	57 ^c	<1	1.0
2-methyl-3-(methylthio)furan	0.4 ^c	<1	1.5
δ -octalactone	2490 ^e	<1	<1
2,3,5-trimethylpyrazine	290 ^d	<1	3.2
1-octen-3-one	10 ^c	<1	<1
δ -decalactone ^b	590 ^d	<1	<1
(E,E)-2,4-nonadienal	2500 ^c	<1	<1
2-acetyl-1-pyrroline	0.1 ^c	nd	42

^a Orthonasal odor thresholds determined in sunflower oil. ^b OAVs were calculated by dividing the concentration of an odorant by its orthonasal detection threshold; nd, not determined. ^c Threshold values according to ref 44. ^d Threshold values according to ref 23. ^e Schmitt and Schieberle, unpublished results.

(2660), 3-methylbutanal (2610) and phenylacetaldehyde (250) as well as 3-methylbutanoic acid (440) and ethyl 2-methylbutanoate (135) showed the highest odor activity in the roasted beans. In addition, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like) and 2-acetyl-1-pyrroline (popcorn-like), which were not present above their odor thresholds in the unroasted cocoa, showed quite high OAVs in the roasted seed. Among the pyrazines, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine showed the highest OAVs, although it can be assumed that the four alkylpyrazines show additive effects in the overall aroma because of their very similar odor quality.

Correlation of Odor Activity Values and Results of the Aroma Profile Analysis. The changes in the overall aroma profile from unroasted to roasted Criollo cocoa beans can easily be explained on the basis of the changes in OAVs caused by the roasting process. The aroma of unroasted Criollo beans is dominated by short-chain carboxylic acids, especially acetic acid and 3-methylbutanoic acid. Acetic acid is formed during the fermentation of cocoa beans by enzymatic degradation of the pulp (45) and enters the cocoa beans via diffusion. Concentrations of up to 11.8 g/kg acetic acid have been reported depending upon the fermentation process (46). Although in this study, only 1.1 g/kg of acetic acid were found, its impact on the aroma of unroasted cocoa beans was corroborated by the fact that the sour, vinegar-like odor quality was ranked as the most intense (Figure 1). During the roasting process, the concentration of acetic acid was decreased by about 70% and, thus, the aroma of roasted cocoa beans was no longer dominated by a vinegar-like note (Figure 1). On the other hand, the roasting process

had only minor influence on the concentrations of the rancid smelling 3-methylbutanoic acid (**Table 3**). Ziegleder and Biehl (18) had proposed that 3-methylbutanoic acid is formed at the end of the fermentation from the amino acid leucine by aerobic putrefactive bacteria. The esters ethyl 2-methylbutanoate, ethyl 2-methylpropanoate, and 2-phenylethyl acetate were present in similar concentrations in the unroasted as well as roasted cocoa beans (**Table 3**). The esters might be present already in the unfermented beans but may also be generated during fermentation. Interestingly, no losses occurred during roasting.

The largest impact on the aroma caused by the roasting process was observed for the Strecker aldehydes 3-methylbutanal (malty) and phenylacetaldehyde (honey-like), as well as for 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), the concentrations of which were significantly higher in the roasted beans. These results were well-correlated with the data of the aroma profile analyses, indicating that the malty and caramel-like qualities of these compounds were also rated clearly higher in the unroasted cocoa beans (**Table 3** and **Figure 1**). A contribution of 3-methylbutanal to the aroma of cocoa and cocoa products has previously been proposed by numerous authors (3, 11, 47, 48), but its key role for the aroma of cocoa mass or cocoa powder has only previously been confirmed (9, 23). Furthermore, although the formation pathway leading to 3-methylbutanal and phenylacetaldehyde, respectively, has been suggested by a Strecker degradation of the parent amino acids leucine and phenylalanine in many studies, no clear quantitative correlation is yet available between the amounts of the precursors degraded and the amounts of the odorants formed in model experiments using the "natural" concentrations of these precursors. Furthermore, as very recently found by us (Weigl and Schieberle, unpublished) besides the free amino acids, also the Amadori products and certain dipeptides act as potent precursors of the Strecker aldehydes.

4-Hydroxy-2,5-dimethyl-3(2H)-furanone, an odorant present in many thermally processed foods, such as coffee, meat, or bread crust, was first described as cocoa constituent by Ziegleder (49). Semi-quantitative determinations had revealed concentrations of <0.1 mg/kg for unroasted and 0.5–2 mg/kg for roasted cocoa beans, which were in the same concentration range as reported in **Table 3**. Besides a thermal degradation of glucose or fructose, 4-hydroxy-2,5-dimethyl-3(2H)-furanone can more effectively be formed in a thermal degradation of either rhamnose or fructose-1,6-biphosphate (50). However, it is yet unclear whether these precursors do exist in cocoa beans.

In general, our results suggest that the differences in the aroma profiles of unroasted and roasted Criollo beans are caused by quantitative rather than a qualitative change in the composition of only a quite small set of key aroma compounds. Of the 30 aroma compounds quantified, only acetic acid was significantly reduced by the roasting process, while 15 compounds increased in their concentrations by more than a factor of 2. Most compounds, however, were scarcely influenced by the heat treatment. The results of our study might also explain why an experiment by Lopez and Quesnel (48) to create the characteristic aroma of roasted cocoa beans by thermally reacting reducing sugars and amino acids was not successful, because important compounds in the typical roasted cocoa aroma, such as acids, esters, and alcohols, are not formed from precursors during the roasting process but are delivered by the fermented unroasted cocoa bean. Further studies to clarify which of the odorants are already present in the unfermented cocoa beans are currently under investigation.

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