Decoding the Fine Flavor Properties of Dark Chocolates

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Abstract

Fine flavor properties of chocolates such as fruity, floral and cocoa-like were decoded on a molecular level for the first time. The molecular compositions of six chocolates made out of liquors that were referenced with specific sensory attributes were analyzed. After the screening for odor-active molecules by aroma extract dilution analysis, selected compounds were quantitated with the overall aim to decode the distinct fine flavor attributes on a molecular level. Acidic and fruity flavor notes were associated with high dose over threshold factors (DoT factors) of acetic acid and fruity smelling esters such as ethyl 2-methylbutanaote, ethyl 3-methylbutanoate and 3-methylbutyl acetate, respectively. Cocoa-like and roasty flavor notes were associated with high DoT factors for 2-methylbutanal, 3-methylbutanal, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane. The floral and astringent flavor was linked to high DoT factors of (−)-epicatechin, procyanidin B2, procyanidin C1 and 2-phenylethan-1-ol.

Keywords

Theobroma cacao, fine flavors, sensory references, dark chocolates, molecular flavor compositions, stable isotopically substituted odorants

Introduction

Chocolate is a popular food and consumed all over the world due to its unique flavor and texture.[1] Recent developments show a higher demand of consumers for high quality chocolate, organic cocoa and bean-to-bar products.[2] Bean-to-bar chocolates are made from fine or flavor cocoa of defined origin and variety and differ in their sensory properties from those of chocolates which are produced on high industrial scale.[3] Industrially produced chocolates require a consistent, standard quality which is usually achieved by blending cocoa beans of bulk quality from different origins.[4]

As a result of the mentioned developments, the diversity of chocolate flavor profiles on the market is increasing. In parallel, the importance of global standards for assessing the flavor quality of cocoa and chocolate is rising. The development of such standards including sensory evaluation protocols[5] is done by a working group that is coordinated by the Cocoa of Excellence (CoEx) program.[6] The CoEx program recognizes cocoa quality and flavor diversity, celebrates unique origins and rewards cocoas with unique flavors.[7] Within this program, cocoa beans from all over the world are evaluated and the best 50 chocolates are awarded after a professional sensory evaluation of both, liquors and chocolates.[8] Within the great diversity of cocoa samples, certain liquors were identified as suitable as reference samples for specific sensory attributes due to their very distinct flavor profiles. Such references are important for the training of sensory panels and essential for a global, standard sensory assessment of cocoa and chocolate.

While the sensory diversity of cocoa products, especially from defined origins and varieties, is widely described in the literature,[4,9–11] the molecular background is not fully understood yet. Fine flavor attributes which are described include fruity, floral, acidic and cocoa[9,12] and are mostly based on sensory evaluations. In contrast to that, the flavor development along the cocoa processing chain has been well studied on the chemical level[1] and the odor-active compounds in cocoa and chocolate have been analyzed in several studies.[13–15] However, most studies with focus on sensory-active compounds analyzed cocoa products with no defined origin and flavor characteristics.[13,15,16] Cocoa products with no defined origin are usually blends from bulk grade cocoa beans and differ significantly in their sensory properties from those of single-origin chocolates.[14] While off-flavors like smoky[17] and moldy-musty[18] as well as a specific coconut-like odor[19] in cocoa have been elucidated on a molecular level, flavor-active compounds that are responsible for specific fine flavor properties still have to be identified. Differences between fine or flavor and bulk cocoa could be found in their volatile composition but these studies did not focus on sensory-active compounds.[20,21]

First attempts to analyze the molecular background of cocoa products with different flavor qualities by a combination of instrumental analysis and sensory methods were made in several studies. Deuscher et al.[22] analyzed dark chocolates that were grouped according to their sensory properties with gas chromatography-olfactometry (GC-O). As a results, they found certain odorants associated with the four different groups. Liu et al.[23] analyzed two chocolates and a cocoa liquor with the focus on odor-active compounds perceived during GC-O analysis. They found a correlation of a malty odor perceived during sensory evaluation with high concentrations of 2-methylpropanal, 3-methylbutanal and volatile carboxylic acids. Phenylacetaldehyde and 2-phenylethan-1-ol were assumed to be responsible for a floral odor, but no compounds could be associated with a fruity odor. Rottiers et al.[24] analyzed liquors of four EET cultivars and one CCN51 sample and did both, a sensory characterization and a semi-quantitation of volatiles with headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). Based on the obtained odor activity values, they suggested a broad range of compounds to be responsible for fruity, floral, chocolate/nutty and buttery/creamy odors.

Even though these attempts provide valuable information, methods including aroma extract dilution analysis (AEDA) combined with the quantitation of odorants by gas chromatography-mass spectrometry (GC-MS) using isotopically substituted odorants as internal standards are necessary to fully elucidate the molecular background of specific flavor attributes. Such methods have been applied to Nacional cocoa samples which were characterized by more intense floral, honey-like and malty odor notes in comparison to CCN51 samples.[25] Additionally, non-volatile taste-active compounds have an impact on the flavor properties and have to be analyzed as well. Samples that are distinct in their flavor attributes like the CoEx reference samples have the potential to provide valuable insights into the chemical signature of flavor-active compounds that have to be present to evoke specific flavor perceptions. To the best of our knowledge, no comprehensive study has decoded fine flavor attributes like fruity, floral and cocoa-like with the above mentioned techniques. Therefore, the aim of our study was to decode those fine flavor properties in dark chocolates on a molecular level. The analyzed chocolates were made out of CoEx sensory reference liquors with flavor attributes such as fruity and acidic, cocoa-like and roasty as well as floral and astringent. After an aroma extract dilution analysis of three chocolates, selected odorants were quantitated in six chocolates by means of gas chromatography-mass spectrometry using stable isotopically substituted odorants as internal standards. Additionally, important cocoa tastants as known from literature[26] were quantitated.

Materials and Methods

Chocolates

The six reference chocolates were produced out of the respective reference liquors and kindly provided by CoEx. The chocolates included three references described with an intense fruity flavor, two chocolates with a distinct cocoa-like flavor, and one chocolate characterized by intense floral and astringent notes. The reference attributes and further data are listed in Table 1. The dark chocolates were produced with 25% sugar. The roasting protocols can be found in the Supporting Information (Table S1).

Odorants

The reference odorants 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, 2-methylbutanoic acid, 3-methylbutanoic acid, acetic acid, phenylacetic acid, 3-methylbutyl acetate, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl phenylacetate, 2-phenylethyl acetate, ethyl (2*E*)-3-phenylprop-2-enoate, linalool, 2-phenylethan-1-ol, 2,3-diethyl-5-methylpyrazine, 2-ethyl-3,(5 or 6)-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2-methoxyphenol, 4-methylphenol, 2-methyl-3-(methyldisulfanyl)furan, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, dimethyltrisulfane, γ-decalactone, γ-nonalactone, vanillin, ethyl 2-methylpropanoate, butane-2,3-dione, methyl butanoate, ethyl butanoate, oct-1-en-3-one, 3-(methylsulfanyl)propanal, 2-methoxy-3-sec-butylpyrazine, 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-isobutylpyrazine, 2-methylpropanoic acid, (2*E*,6*Z*)-nona-2,6-dienal, butanoic acid, (2*E*,4*E*)-nona-2,4-dienal, ethyl 3-phenylpropanoate, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, 4-ethylphenol, 3-ethylphenol, 4-ethenyl-2-methoxyphenol and 2,6-dimethoxyphenol were purchased from Merck (Darmstadt, Germany). 3-Methylnonane-2,4-dione was purchased from AromaLAB (Planegg, Germany).

The stable isotopically substituted odorants 2-($^{2}H_{3}$)methylbutanal, 3-($^{2}H_{3}$)methyl(3,4,4,4$^{2}H_{4}$)butanal, phenyl($^{13}C_{2}$)acetaldehyde, 3-($^{2}H_{3}$)methyl(2,2,3,4,4,4-$^{2}H_{6}$)butanoic acid, phenyl($^{13}C_{2}$)acetic acid, 3-methylbutyl ($^{13}C_{2}$)acetate, ethyl 2-($^{2}H_{3}$)methylbutanoate, ethyl 3-($^{2}H_{3}$)methyl(2,2,3,4,4,4-$^{2}H_{6}$)butanoate, ethyl ($^{2}H_{5}$)phenylacetate, 2-($^{2}H_{5}$)phenylethyl acetate, ethyl (2*E*)-3-($^{2}H_{5}$)phenyl(2,3-$^{2}H_{2}$)prop-2-enoate, ($^{2}H_{3}$)methyl-7-methyl-(4,4-$^{2}H_{2}$)octa-1,6-dien-3-ol, 2-($^{2}H_{5}$)phenylethan-1-ol, 2-(1,1-$^{2}H_{2}$)ethyl-3(1,1-$^{2}H_{2}$)ethyl-5-($^{2}H_{3}$)methylpyrazine, 3-($^{2}H_{5}$)ethyl-2,5-dimethylpyrazine, 2-($^{3}H_{2}$)methyl-3,5-dimethylpyrazine, 2-($^{2}H_{3}$)methoxyphenol, 4-methyl(2,6-$^{2}H_{2}$)phenol, 2-methyl-3-(($^{2}H_{3}$)methyldisulfanyl)furan, 5-(1,1-$^{2}H_{2}$)hexyl-(3,3,4,4,5-$^{2}H_{5}$)oxolan-2-one, 3-hydroxy-4-methyl-5-(^{13}C)methyl(5-^{13}C)furan-2(5*H*)-one, ($^{2}H_{6}$)dimethyltrisulfane, 4-hydroxy-2-methyl-5-(^{13}C)methyl(5-^{13}C)furan-3(2*H*)-one, 4-hydroxy-3-($^{2}H_{3}$)methoxybenzaldehyde and

5-(4,4,5,5-$^{2}H_2$)pentyloxolan-2-one were purchased from AromaLAB. ($^{13}C_2$)Acetic acid was purchased from Merck.

Miscellaneous Reference Substances

Caffeine, theobromine and (−)-epicatechin were obtained from Merck. Procyanidin B2 and procyanidin C1 were purchased from Phytolab (Vestenbergsgreuth, Germany). Cyclo(D-ala-L-Val) was purchased from Bachem (Bubendorf, Switzerland).

Defatting of Chocolate

Chocolate was defatted according to Pedan et al.[27] with slight modifications. 10 g of chocolate was diluted in 40 mL *n*-hexane (Roth, Arlesheim, Switzerland). The extraction was carried out with a benchtop shaker (Hettich Labtechnology, Tuttlingen, Germany) at 500 rpm and room temperature for 10 min. After centrifugation at 4000 rpm (3220*g*) for 5 min, the hexane phase was removed by decanting. The described extraction process was repeated additional three times (in total four extractions). The procedure was repeated on the day after. The residue was frozen for at least 2 hours at −80 °C and then lyophilized in a freeze dryer (Martin Christ, Osterode am Harz, Germany). It was assumed that only insignificant amounts of the quantitated tastants were removed with the *n*-hexane.

Sample Work-up for Gas Chromatography-Olfactometry

25 g of chocolate was broken into pieces by hand and 60 mL of ultrapure water and 180 mL of diethyl ether (freshly distilled before use) were added in an Erlenmeyer flask. After an extraction for at least 12 hours, the diethyl ether phase of the extract was separated by means of a separating funnel and a centrifuge (10 min, 11000 rpm, 14610*g*) before it was subjected to solvent-assisted flavor evaporation (SAFE).[28] The thawed SAFE distillate was dried over anhydrous sodium sulfate and concentrated up to a volume of 300 µL.

Sample Work-up for Quantitation of Odorants

Chocolate (1 or 20 g) was broken into pieces by hand. Ultrapure water (15 or 50 mL) and diethyl ether (45 or 150 mL) were added subsequently. Water addition was crucial to release additional amounts of odorants from their hydrolabile precursors and thus mimic the retronasal odorant profile perceived during consumption.[29] Stable isotopically substituted odorants (0.002−3313 µg) in diethyl ether (20−400µL) were added in an amount as expected for the target compounds in the sample. The sample was stirred for at least 12 hours with a magnetic stirrer. The diethyl ether phase was then separated by a separating funnel (1 g samples) or by centrifugation according to the protocol detailed before (20 g samples). The diethyl ether phases were subjected to SAFE.[28] The thawed distillate was dried over anhydrous sodium sulfate. The distillate obtained from the 1 g samples was concentrated to a volume of 5−10 mL using a Vigreux column. A small amount was taken for analyzing odorants of high concentrations like acetic acid and the residual distillate was concentrated to a volume of 300 µL using a gentle stream of nitrogen. The distillate obtained from the 20 g samples was completely concentrated to a volume of 300 µL using first the Vigreux column and then a gentle stream of nitrogen.

Sample Work-up for Quantitation of Tastants

All tastants were analyzed in the defatted chocolate powder. Citric acid and lactic acid were extracted from 0.5 g with 5 mL ultrapure water. The mixture was vigorously shaken and then placed in a water bath tempered at 80 °C for 10 min. An aliquot of 2 mL was centrifuged at 15000 rpm (25150*g*) for 10 min and the supernatant was used for analysis. The sample preparation for the quantitation of caffeine, theobromine, (−)-epicatechin, procyanidin B2 and procyanidin C1 was carried out with 1 g according to Pedan et al.[27] The combined supernatants were filtered (pore size: 0.7 µm) before analysis. The sample work-up for the quantitation of cyclo(L-pro-L-val) was performed according to André et al.[30]

Aroma Extract Dilution Analysis

The GC-O system consisted of an Agilent 7890B gas chromatograph (Agilent Technologies, Basel, Switzerland) coupled to an Agilent 5977A MSD mass spectrometer and an olfactory detection port (ODP3) (Gerstel, Mülheim an der Ruhr, Germany). Separation of volatiles was carried out on a DB-FFAP column (30 m length, 0.32 mm inner diameter, 0.25 µm film thickness; Agilent Technologies) with helium (99.9999% purity) as carrier gas and a constant flow of 3 mL/min. 1 µL of the sample was injected on-column and at the end of the column the effluent was split 1:1 to both detectors. The oven was set to 40 °C for 4 min and was then heated to 240 °C at 5 °C/min. Both transfer lines were heated to 250 °C and the mixing chamber of the olfactory detection port was heated to 150 °C. The MS was operated in EI mode with an ionization energy of 70 eV and an ion source temperature of 230 °C. Chromatograms were recorded in scan mode with a range of 50−250 *m/z*.

The AEDA was carried out with the samples Ref1, Ref4 and Ref5. The concentrated distillates of the samples were diluted stepwise with diethyl ether at a ratio of 1:4 in order to obtain dilutions up to 1:4096. The undiluted and diluted samples were analyzed by GC-O in order to obtain flavor dilution (FD) factors for all odor-active compounds.[31] Identification of the odor-active compounds was done by comparison of retention index (RI), odor quality and mass spectrum to data obtained from the analysis of reference compounds and from the literature. Retention indices were additionally determined on a DB-5 column (30 m length, 0.32 mm inner diameter, 0.25 µm film thickness; Agilent Technologies) using the parameters described above but a final temperature of 270 °C instead of 240 °C.

Quantitation of Odorants by Gas Chromatography-Mass Spectrometry

Depending on the target compound, the quantitation was done either with a GC-MS system or with a GC-GC-MS system. Details are provided in the Supporting Information (Table S2). Both systems were described previously.[29] The method parameters were the same with the following exceptions. The cold trap in the second oven of the GC-GC-MS system was made in-house and was cooled by a nitrogen stream to approximately −120 °C between 3 min before the cut and 0.1 min after the cut.

Quantitation of Lactic Acid and Citric Acid

The quantitation of lactic acid and citric acid was done enzymatically by using kits obtained from r-biopharm (Darmstadt, Germany) in combination with a Chemwell 2910 Automated EIA and Chemistry Analyzer (Awareness Technology, Palm City, United States).

Quantitation of Caffeine, Theobromine, (−)-epicatechin, Procyanidin B2 and Procyanidin C1

The quantitation of alkaloids and individual polyphenols was done with an Agilent 1260 Infinity chromatography system equipped with a 1260 diode array detector. The separation was performed at 35 °C using an Agilent Poroshell 120 EC-C18 (4.6 × 100 mm, 2.7 µm) column preceded by a guard column (Agilent EC-18, 2.1 × 5 mm, 2.7 µm). The flow rate was 0.8 mL/min and the mobile phases consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient was as follows: 0−2 min, 5 % B; 4−9 min, 11% B; 11 min, 20% B; 13 min, 24% B; 18 min, 27% B; 20 min, 30% B; 22−30 min, 100% B; 30.1−35 min, 5% B. The injection volume was 2 µL and UV spectra were recorded at 275 nm. The calibration curves were recorded at 275 nm and are listed in Table S3 in the Supporting Information.

Quantitation of Cyclo(L-pro-L-val)

Cyclo(L-pro-L-val) was quantitated with high performance liquid chromatography-mass spectrometry/mass spectrometry (HPLC-MS/MS) and *trans*-cyclo(D-ala-L-val) was used as internal standard according to André et al.[30]

Statistics

The F-test for differences between the six reference chocolates was carried out with a level of significance of α=0.05. Statistical analysis and data visualization were done with Python 3.8.3.

Results and Discussion

Aroma Extract Dilution Analysis of three Reference Chocolates

The concentrated distillates of Ref4, Ref5 and Ref1 were subjected to aroma extract dilution analysis in order to identify the odor-active compounds in the chocolates with three different sensory profiles. Ref1 represented an odor profile dominated by intense cocoa-like and roasty notes. Ref4 represented a flavor profile described as intense fruity and acidic and a floral dominated odor profile was attributed to Ref5. All odor-active compounds that were detected in at least two samples and in at least one of the samples with an FD factor of 16 are listed in Table 2. From 50 odor-active compounds, 47 could be identified and 3 remained unknown.

The highest FD factors in Ref1 (cocoa, roasty) of 1024 or 4096 were found for acetic acid, 2,3-diethyl-5-methylpyrazine, 2- and 3-methylbutanoic acid, dimethyltetrasulfane, 2-methoxyphenol, 2-phenylethan-1-ol, ethyl cinnamate, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one and phenylacetic acid. Higher FD factors in Ref1 (cocoa, roasty) compared to Ref4 (fruity, acidic) and Ref5 (floral, astringent) were obtained for 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 2,3-diethyl-5-methylpyrazine, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol and 2-methoxy-4-vinylphenol. Additionally, Ref1 (cocoa, roasty) showed high FD factors for most of the pyrazines and phenols. 2- and 3-Methylbutanal, which have been proven to be important key odorants in cocoa and chocolate,[14–16,32] were perceived up to an FD factor of 64 in Ref1. These compounds were also perceived up to an FD factor of 64 in Ref5 (floral, astringent) and up to an FD factor of 16 in Ref4 (fruity, acidic). Many odorants described with sulfury notes such as dimethyltetrasulfane, dimethyltrisulfane or a seasoning-like odor quality such as 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one showed as well higher FD factors in Ref1 (cocoa, roasty) than in Ref4 (fruity, acidic) and Ref5 (floral, astringent).

In Ref 5 (floral, astringent), 2- and 3-methylbutanoic acid and ethyl cinnamate were perceivable with the highest FD factor of 1024. Interestingly, most of the odorants in the floral reference were detectable with somewhat lower FD factors than in the other two samples. However, the floral smelling odorants 2-phenylethan-1-ol and 2-phenylethyl acetate both showed FD factors of 256, indicating the importance of these odorants to the intense floral odor.

Ref4 was described as intense fruity and acidic and showed a very high FD factor of 1024 for the vinegar-like smelling acetic acid. Other compounds with an FD factor of 1024 were 2-methyl-3-(methyldisulfanyl)furan, 2-phenylethyl acetate, ethyl 3-phenylpropanoate, 2-phenylethan-1-ol, ethyl cinnamate and phenylacetic acid. Ref4 (fruity, acidic) showed the highest FD factors for many fruity smelling compounds within the three samples except for ethyl butanoate and 3-methylbutyl acetate.

Nearly all of the detected odorants have been previously identified as cocoa odor constituents.[13–18,32] However, the methoxyphenols 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol and 2-methoxy-4-vinylphenol have not been reported in the other studies. All showed smoky odor qualities and have also not been identified in cocoa beans with a smoky off-flavor.[17]

The AEDA results revealed first differences between the samples and allowed the assumption that the characteristic flavor profiles of the reference chocolates were caused by quantitative differences of well-known chocolate key odorants.

Quantitation of Odorants and Tastants in the six Reference Chocolates

Selected odorants were quantitated in all six reference chocolates. The selection was based mainly on the results of the AEDA combined with previous findings on key odorants in the literature. Additionally, important key tastants known from literature[26] were quantitated. Cyclo(L-pro-L-val) was chosen for quantitation as the most important diketopiperazine for a bitter taste in cocoa.[26] The concentrations are listed in Table 3.

The concentrations obtained for the chocolates for acetic acid, 2- and 3-methylbutanoic acid, 2-phenylethan-1-ol, phenylacetic acid, 2- and 3-methylbutanal, 2-phenylethyl acetate, phenylacetaldehyde and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one were mostly higher than in the previous studies.[14,15] This is most likely the result of the water addition before the work-up which releases additional amounts of odorants.[29] Compared to six traditionally manufactured chocolates analyzed by Chetschik et al.,[14] the chocolates analyzed in this study showed predominantly lower concentrations of ethyl phenylacetate and 3-methylbutyl acetate, but higher concentrations of 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, ethyl 2-methylbutanoate and ethyl 3-methylbutanoate. Interestingly, 2-phenylethan-1-ol showed higher concentrations in all six reference chocolates compared to chocolates in previous studies.[14,15]

The quantitation of selected flavor-active compounds revealed more distinct differences between the chocolates than the AEDA. The highest concentrations for acetic acid were found in Ref2, Ref3 and Ref4 – all described as acidic and fruity. The concentrations of 2,3,5-trimethylpyrazine, 2-methoxyphenol and all the quantitated esters were predominantly higher in these three chocolates than in the other three chocolates. The differences were most pronounced for 2-phenylethyl acetate with concentrations of 1610−3220 µg/kg and for ethyl cinnamate with concentrations of 138−402 µg/kg in the three chocolates referenced as fruity and acidic. The two chocolates that were additionally referenced with a browned fruit flavor (Ref2, Ref3) showed higher concentrations of ethyl 3-methylbutanoate and 2-methoxyphenol than Ref4. Lactic acid with 242−641 mg/100 g showed the highest concentrations in the three chocolates described as acidic and fruity (Ref2, Ref3, Ref4) among all samples, while the concentrations of citric acid were not especially high in this group. The concentrations of the Strecker aldehydes 2- and 3-methylbutanal and phenylacetaldehyde as well as 2,3-diethyl-5-methylpyrazine, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane were highest in the two chocolates described as cocoa and roasty (Ref1, Ref6). Although both chocolates were referenced as distinct cocoa-like and roasty, Ref1 showed higher concentrations for most odorants than Ref6 except for acetic acid, ethyl 2-methylbutanoate and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one. Additionally, the concentrations of the bitter tasting compounds theobromine, caffeine and cyclo(L-pro-L-val) were higher in Ref1 than in Ref6, whereas the three quantitated polyphenols were present at higher concentrations in Ref6. The concentration of cyclo(L-pro-L-val) with 13.7 mg/100 g was clearly the highest among all samples in Ref1 (cocoa, roasty). Ref5 (floral, astringent) showed remarkably higher concentrations of (−)-

epicatechin, procyanidin B2 and procyanidin C1 than the other five chocolates. Furthermore, the highest concentrations of 2-phenylethan-1-ol with 8650 μg/kg were found in Ref 5 (floral, astringent) and Ref3 (fruity, acidic).

Decoding of the Fine Flavor Attributes in the Reference Chocolates

The concentrations of the flavor-active compounds revealed differences in the molecular compositions of the chocolates with the different sensory profiles. However, the impact on the sensory perception cannot be concluded from the concentrations alone. The ratios of the concentrations to their odor or taste thresholds have to be calculated in order to assess the contribution of the odorants and tastants to the overall odor and taste perception. This ratio is often expressed as odor activity value (OAV) for odorants[33] and dose over threshold factor (DoT factor) for tastants.[34] As both, OAVs and DoT factors are calculated as the ratio of concentration to odor or taste threshold, the term DoT factor is used for both, odorants and tastants in the following. All DoT factors are listed in Table 4. Acetic acid showed by far the highest DoT factors of >4000 in all samples, followed by 3-methylbutanoic acid, dimethyltrisulfane, phenylacetic acid and 3-methylbutanal. Acetic acid showed the highest DoT factors among the sour tasting compounds and the highest DoT factors among the bitter tasting compounds were observed for theobromine. Procyanidin B2 showed the highest DoT factors for an astringent perception. The DoT factors were applied to a principal component analysis (PCA) (Figure 1). 2-Phenylethyl acetate, ethyl cinnamate, γ-decalactone, γ-nonalactone and cyclo(L-pro-L-val) were excluded as DoT factors were <1 in all samples and these compounds were not assumed to be relevant in explaining the different flavor profiles. Principal components (PC) 1 and PC 2 explained 65.76% of the variance in total. The PCA of the molecular flavor compositions separated the six samples into three clusters as indicated with the red circles. Samples with similar sensory properties were clustered together which suggested that the key compounds responsible for the different flavor profiles were included in the PCA.

The first cluster consisted of the three chocolates Ref2, Ref3, Ref4. All were referenced with an intense fresh fruit odor and a high acidity. The fruity odor of Ref2 and Ref3 was additionally described as browned fruit. The negative values on PC1 and PC2 of the chocolates in this cluster were associated with high DoT factors of the fruity smelling esters ethyl 2-methylbutanoate, ethyl 3-methylbutanoate and 3-methylbutyl acetate. Mostly higher DoT factors of these compounds were found in all chocolates described as distinct fruity and acidic (Ref2, Ref3, Ref4) compared to the other samples which corresponds to the intense fruity odor. While the DoT factor of the banana-like smelling ester 3-methylbutyl acetate was highest in Ref4 (3.64) followed by Ref3 (3.02), the DoT factor was below 1 in Ref2. Ref2 showed the highest DoT factor for ethyl 2-methylbutanoate (8.18) and Ref3 showed the highest DoT factor for ethyl 3-methylbutanoate (3.82). Therefore, it could be assumed that none of these compounds alone was responsible for the distinct fruity odor. Instead, rather the combination of all fruity smelling esters contributed to the fruity odor perception. Interestingly, the DoT factors of 2-methoxyphenol and 2,3,5-trimethylpyrazine were highest in the samples within this cluster even though 2,3,5-trimethylpyrazine, as a roasty smelling compound, would be expected to be higher in the samples with the roasty odor (Ref1, Ref6). Ref2 and Ref3 were additionally described with a browned fruit character (Table 1). While the general fruitiness of the samples could be well explained by higher DoT factors of fruity smelling esters and acetic acid, specifications like browned fruits were more difficult to elucidate on a molecular level. Compounds that were described with an odor quality of dried fruits during AEDA were 2-phenylethanol and 2-phenylethyl acetate. Ref3 showed a high DoT factor of 17.6 for 2-phenylethan-1-ol which was not significantly lower than the highest one of Ref5 (floral, astringent). Additionally, the concentration of 2-phenylethyl acetate was highest in Ref3. Even though the DoT factors of 2-phenylethyl acetate were <1 in all chocolates, this compound could have an additive effect for a browned fruit odor even at subthreshold concentrations. Such effects have not been studied in a complex matrix like chocolate, but were shown for the fruity odor of wine.[35] However, Ref2 was as well described as browned fruit but with 9.03 showed the lowest DoT factor for 2-phenylethan-1-ol among all samples and a lower concentration of 2-phenylethyl acetate than Ref 4 in which the browned fruit character could not be detected. Interestingly, both Ref2 and Ref3 showed significantly higher DoT factors of ethyl 3-methylbutanoate and 2-methoxyphenol than Ref4. In addition, significantly lower DoT factors of acetic acid were observed in Ref2 and Ref3 compared to Ref4. With the highest DoT factors in all samples, acetic acid was supposed to have a major impact on the sensory perception. The highest DoT factor for acetic acid among all samples in Ref4 suggested a more intense sour perception compared to Ref2 and Ref3. This sour perception may have influenced the fruity odor perception in Ref4 in a way that the fruity odor was perceived as intense fresh fruits-like. The browned fruits odor notes were perceived less distinctly in Ref4 than in Ref2 and Ref3 which showed lower DoTs factors of acetic acid. Rottiers et. al.[24] already suggested several esters to be responsible for fruity odor notes. They further suggested linalool and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one to play a role for a fruity odor in cocoa liquor. However, these two compounds were not associated with fruity dominated flavor profiles in our study. The importance of esters for a fruity flavor could be confirmed by our data with the highest impact of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate and 3-methylbutyl acetate.

The significantly highest DoT factors of acetic acid in the three chocolates referenced as fruity and acidic correspond to their intense acidic sensory properties. Acetic acid was assumed as the most impactful contributor to the acidity due to its highest DoT factors. Acetic acid contributed to the pungent, vinegar-like odor and the sour taste perception. However, its taste threshold[26] is 343 times higher than its odor threshold.[36] In addition to acetic acid, citric acid and lactic acid can impact the acidity by their sour taste. The highest DoT factors of lactic acid among all samples were found in Ref2, Ref3 and Ref4. Finally, it can be assumed that the acidic flavor was evoked by acetic acid in combination with lactic acid and citric acid. However, this observation is yet to be confirmed by sensory experiments.

Another cluster was formed by Ref1 and Ref6. Both were described as distinct cocoa-like and roasty. The high PC1 values of the samples were linked to high DoT factors of a number of odorants. None of the individual odorants was described as typical cocoa-like during AEDA. Therefore, it can be assumed that a combination of odorants was responsible for creating the cocoa-like odor. Ref1 and Ref6 showed the highest DoT factors for the Strecker aldehydes 2-methylbutanal, 3-methylbutanal and phenylacetaldehyde. Furthermore, they showed the significantly highest DoT factors of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane. The DoT factors of phenylacetic acid, 2- and 3-methylbutanoic acid and 2-methyl-3-(methyldisulfanyl)furan were as well very high in Ref1 and Ref6 compared to the other chocolates. Interestingly, the DoT factors of the roasty smelling pyrazines were not especially high in these samples. The DoT factor of 2,3-diethyl-5-methylpyrazine was highest in Ref1 compared to the other samples, but with 1.14 relatively low and even below 1 in Ref6. Low DoT factors of pyrazines even in roasted cocoa were already found by Frauendorfer and Schieberle[16] who were the first to question the importance of the pyrazines to the overall cocoa flavor.

The third cluster was represented by Ref5 (floral, astringent) which was well separated from the other two clusters. The floral odor of Ref5 was probably mainly caused by 2-phenylethan-1-ol as this floral smelling compound in this sample with 17.7 showed the highest DoT factor among all samples followed by Ref3 (fruity, acidic). Ref5 did not show especially high DoT factors of other floral smelling odorants like linalool, phenylacetaldehyde and phenylacetic acid compared to the other five chocolates. The DoT factor of 2-phenylethan-1-ol was similar in Ref3 (fruity, acidic) and the DoT factors of the other floral smelling odorants were higher in Ref1 and Ref6 (cocoa, roasty) although these chocolates were not described as distinctly floral. However, the DoT factors of other chocolate key odorants like dimethyltrisulfane and 3-methylbutanal were low in Ref5 and the relatively high DoT factors of 791 for phenylacetic acid, 53.7 for phenylacetaldehyde and 36.6 for linalool indicated that these odorants contributed to the overall floral flavor perception. Consequently, interactions during the perception of the flavor-active compounds seem to be important and the specific combination of concentrations of floral smelling odorants together with other key odorants may have caused the distinct floral flavor profile. The suggestion that linalool is mainly responsible for floral notes in chocolate could not be confirmed by our data.[37] Ref5 showed a DoT factor of 36.6 for linalool which was not especially high compared to the other analyzed chocolates. The important role of 2-phenylethan-1-ol for a floral odor in cocoa and chocolate was suggested previously[23–25,38] and could be confirmed by our data. Floral and honey-like odor notes in Nacional samples were previously associated with additionally higher concentrations of phenylacetaldehyde, linalool, 2-phenylethyl acetate and ethyl phenylacetate compared to CCN51 samples.[25] The DoT factors of these compounds were not especially high in Ref5 (floral, astringent) compared to the other chocolates analyzed in our study. Ref5 was additionally characterized by the significantly highest DoT factors of (−)-epicatechin, procyanidin B2 and procyanidin C1 among all samples. It can be assumed that these compounds are responsible for the distinct astringent perception in this sample with a DoT factor of 17.7 for (−)-epicatechin, 20.9 for procyanidin C2 and 6.96 for procyanidin C1. These compounds, especially (−)-epicatechin, can additionally enhance the bitter perception.[26] Furthermore, this sample showed the second highest DoT factor of 78.6 for theobromine among all samples. Interestingly, the diketopiperazine cyclo(L-pro-L-val) showed a very low impact on the bitter perception with DoT factors of <1 in all samples. Consequently, theobromine has the highest impact on the bitter taste as already determined by Stark et al.[26]

Although different studies elucidated the odor of cocoa and chocolate on a molecular level,[13–15] the molecular background of specific fine flavor attributes such as fruity, floral and cocoa-like in chocolate has not been fully decoded. Our study showed for the first time how distinct differences in the flavor profiles of dark chocolates are reflected in the molecular compositions. Additionally, flavor-active compounds that are most likely responsible for those sensory attributes were identified. High DoT factors of acetic acid and fruity smelling esters such as ethyl 2-methylbutanaote, ethyl 3-methylbutanoate and 3-methylbutyl acetate are assumed to be responsible for fruity and acidic notes. The DoT factor of acetic acid may influence the fruity perception regarding a specification to fresh fruit or browned fruit. High DoT factors of the cocoa key odorants 2-methylbutanal, 3-methylbutanal, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane are suggested to be indicators for a distinct cocoa-like and roasty flavor. Our data further suggest that floral dominated flavor profiles are predominantly linked to a high DoT factor of the floral smelling compound 2-phenylethan-1-ol. An intense astringent and bitter perception is assumed to be caused by high DoT factors of (−)-epicatechin, procyanidin B2 and procyanidin C1 together with a high DoT factor of theobromine.

The results of this investigation constitute a basis for future quality assessment of cocoa and dark chocolates and the optimization of the flavor properties based on raw material selection and processing. Nevertheless, additional cocoa products of different origins and cultivars have to be investigated to fully understand the interplay of the different flavor molecules for the generation of the fine flavor cocoa attributes on the molecular level.

Abbreviations used

AEDA, aroma extract dilution analysis; CoEx, Cocoa of Excellence; FD, flavor dilution; DoT, dose over threshold; GC-MS, gas chromatography-mass spectrometry; GC-O, gas chromatography-olfactometry; HPLC-MS/MS, high performance liquid chromatography-mass spectrometry/mass spectrometry; HS-SPME-GC-MS, headspace-solid phase microextraction-gas chromatography-mass spectrometry; OAV, odor activity value; PC, principal component; PCA, principal component analysis; RI, retention index; SAFE, solvent-assisted flavor evaporation

Nomenclature

caffeine, 1,3,7-trimethyl-3,7-dihydro-1*H*-purine-2,6-dione; citric acid, 2-hydroxypropane-1,2,3-tricarboxylic acid; cyclo(L-pro-L-val), (3*S*,8a*S*)-3-(propan-2-yl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione; γ-decalactone, 5-hexyloxolan-2-one; (−)-epicatechin, (2*R*,3*R*)-2-(3,4-

dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol; ethyl cinnamate, ethyl (2*E*)-3-phenylprop-2-enoate; lactic acid, 2-hydroxypropanoic acid; linalool, 3,7-dimethylocta-1,6-dien-3-ol; γ-nonalactone, 5-pentyloxolan-2-one; procyanidin B2, (2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-8-[(2*R*,3*R*,4*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2*H*-1-benzopyran-4-yl]-3,4-dihydro-2*H*-1-benzopyran -3,5,7-triol; procyanidin C1, (2*R*,3*R*,4*S*)-2-(3,4-dihydroxyphenyl)-4-[(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2*H*-1-benzopyran-8-yl]-8-[(2*R*,3*R*,4*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-3,4-dihydro-2*H*-1-benzopyran-4-yl]-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol; theobromine, 3,7-dimethyl-3,7-dihydro-1*H*-purine-2,6-dione; vanillin, 4-hydroxy-3-methoxybenzaldehyde

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Supporting Information Description

Table S1. Roasting Parameters of the Reference Chocolates

Table S2. Stable Isotopically Substituted Odorants and Parameters used in the Quantitation of Odor-Active Compounds

Table S3. Parameters used in the Quantitation of Taste-Active Compounds

References

(1) Ziegleder, G. Flavour Development in Cocoa and Chocolate. In *Beckett's Industrial Chocolate Manufacture and Use,* 5th ed.; Beckett, S. T., Fowler, M. S., Ziegler, G. R., Eds.; John Wiley & Sons: Chichester, U.K., 2017; pp 185–215.

(2) International Cocoa Organization. Fine or Flavour Cocoa. https://www.icco.org/growing-cocoa/ (accessed December 2021).

(3) The small batch project. What is small batch bean-to-bar chocolate? https://thesmallbatchproject.ch/blogs/news/what-is-small-batch-bean-to-bar-chocolate (accessed December 2021).

(4) Fowler, M. S.; Coutel, F. Cocoa Beans: from Tree to Factory. In *Beckett's Industrial Chocolate Manufacture and Use,* 5th ed.; Beckett, S. T., Fowler, M. S., Ziegler, G. R., Eds.; John Wiley & Sons: Chichester, U.K., 2017; pp 9–49.

(5) International Standards for the Assessment of Cocoa Quality and Flavour. Protocols. https://www.cocoaqualitystandards.org/protocols-for-review (accessed April 2022).

(6) International Standards for the Assessment of Cocoa Quality and Flavour. Initiative background. https://www.cocoaqualitystandards.org/initiative-background (accessed December 2021).

(7) Bioversity International. What is the Cocoa of Excellence Programme? http://www.cocoaofexcellence.org/about-us (accessed December 2021).

(8) Bioversity International. The Cocoa of Excellence Process. http://www.cocoaofexcellence.org/about-us/how-the-cocoa-of-excellence-programme-works# (accessed December 2021).

(9) Afoakwa, E. O.; Paterson, A.; Fowler, M.; Ryan, A. Flavor formation and character in cocoa and chocolate: a critical review. *Critical Reviews in Food Science and Nutrition.* **2008**, *48,* 840–857.

(10) Sukha, D. A.; Butler, D. R.; Umaharan, P.; Boult, E. The use of an optimised organoleptic assessment protocol to describe and quantify different flavour attributes of cocoa liquors made from Ghana and Trinitario beans. *Eur Food Res Technol.* **2008**, *226,* 405–413.

(11) Amores, F.; Butler, D.; Ramos, G.; Sukha, D.; Espin, S.; Gomez, A.; Zambrano, A.; Hollywood, N.; van Loo, R.; Seguine, E. *Project to determine the physical, chemical and*

organoleptic parameters to differentiate between fine and bulk cocoa. Project Completion Report International Cocoa Organization: London, 2007.

(12) International Cocoa Organization. Growing Cocoa. https://www.icco.org/fine-or-flavor-cocoa/ (accessed December 2021).

(13) Frauendorfer, F.; Schieberle, P. Identification of the key aroma compounds in cocoa powder based on molecular sensory correlations. *J. Agric. Food Chem.* **2006**, *54,* 5521–5529.

(14) Chetschik, I.; Pedan, V.; Chatelain, K.; Kneubühl, M.; Hühn, T. Characterization of the flavor properties of dark chocolates produced by a novel technological approach and comparison with traditionally produced dark chocolates. *J. Agric. Food Chem.* **2019**, *67,* 3991–4001.

(15) Seyfried, C.; Granvogl, M. Characterization of the key aroma compounds in two commercial dark chocolates with high cocoa contents by means of the sensomics approach. *J. Agric. Food Chem.* **2019**, *67,* 5827–5837.

(16) Frauendorfer, F.; Schieberle, P. Key aroma compounds in fermented Forastero cocoa beans and changes induced by roasting. *Eur. Food Res. Technol.* **2019**, *245,* 1907–1915.

(17) Füllemann, D.; Steinhaus, M. Characterization of odorants causing smoky off-flavors in cocoa. *J. Agric. Food Chem.* **2020**, *68,* 10833–10841.

(18) Porcelli, C.; Neiens, S. D.; Steinhaus, M. Molecular background of a moldy-musty off-flavor in cocoa. *J. Agric. Food Chem.* **2021**, *69,* 4501–4508.

(19) Porcelli, C.; Steinhaus, M. Molecular characterisation of an atypical coconut-like odour in cocoa. *Eur Food Res Technol.* **2022,** 1–11.

(20) Tuenter, E.; Delbaere, C.; Winne, A. de; Bijttebier, S.; Custers, D.; Foubert, K.; van Durme, J.; Messens, K.; Dewettinck, K.; Pieters, L. Non-volatile and volatile composition of West African bulk and Ecuadorian fine-flavor cocoa liquor and chocolate. *Food Res. Int.* **2020**, *130,* 108943.

(21) Kadow, D.; Bohlmann, J.; Phillips, W.; Lieberei, R. Identification of main fine or flavour components in two genotypes of the cocoa tree (Theobroma cacao L.). **2013,** 90–98.

(22) Deuscher, Z.; Gourrat, K.; Repoux, M.; Boulanger, R.; Labouré, H.; Le Quéré, J.-L. Key aroma compounds of dark chocolates differing in organoleptic properties: A GC-O comparative study. *Molecules.* **2020**, *25,* 1809.

(23) Liu, J.; Liu, M.; He, C.; Song, H.; Guo, J.; Wang, Y.; Yang, H.; Su, X. A comparative study of aroma-active compounds between dark and milk chocolate: relationship to sensory perception. *J. Sci. Food Agric.* **2015**, *95,* 1362–1372.

(24) Rottiers, H.; Tzompa Sosa, D. A.; Lemarcq, V.; Winne, A. de; Wever, J. de; Everaert, H.; Bonilla Jaime, J. A.; Dewettinck, K.; Messens, K. A multipronged flavor comparison of Ecuadorian CCN51 and Nacional cocoa cultivars. *Eur Food Res Technol.* **2019**, *245,* 2459–2478.

(25) Forschungskreis der Ernährungsindustrie e.V. (FEI). Evaluierung chemisch-analytischer und molekularbiologischer Methoden zur Differenzierung von hochwertigem Arriba Edelkakao und Konsumkakao (CCN51) (in German). https://www.fei-bonn.de/download/aif-16796-n.projekt (accessed June 2022).

(26) Stark, T.; Bareuther, S.; Hofmann, T. Molecular definition of the taste of roasted cocoa nibs (Theobroma cacao) by means of quantitative studies and sensory experiments. *J. Agric. Food Chem.* **2006**, *54,* 5530–5539.

(27) Pedan, V.; Fischer, N.; Rohn, S. Extraction of cocoa proanthocyanidins and their fractionation by sequential centrifugal partition chromatography and gel permeation chromatography. *Anal. Bioanal. Chem.* **2016**, *408,* 5905–5914.

(28) Engel, W.; Bahr, W.; Schieberle, P. Solvent assisted flavour evaporation - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices. *Eur. Food Res. Technol.* **1999**, *209,* 237–241.

(29) Ullrich, L.; Neiens, S.; Hühn, T.; Steinhaus, M.; Chetschik, I. Impact of water on odor-active compounds in fermented and dried cocoa beans and chocolates made thereof. *J. Agric. Food Chem.* **2021**, *69,* 8504–8510.

(30) André, A.; Casty, B.; Ullrich, L.; Chetschik, I. Use of Molecular Networking to identify 2,5-diketopiperazines in chocolates as potential markers of bean variety. *HELIYON*, **2022**. https://doi.org/10.1016/j.heliyon.2022.e10770

(31) Schieberle, P.; Grosch, W. Evaluation of the flavour of wheat and rye bread crusts by aroma extract dilution analysis. *Z Lebensm Unters Forsch.* **1987**, *185,* 111–113.

(32) Frauendorfer, F.; Schieberle, P. Changes in key aroma compounds of Criollo cocoa beans during roasting. *J. Agric. Food Chem.* **2008**, *56,* 10244–10251.

(33) Rothe, M.; Thomas, B. Aromastoffe des Brotes. *Z Lebensm Unters Forsch.* **1963**, *119,* 302–310.

(34) Scharbert, S.; Hofmann, T. Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *J. Agric. Food Chem.* **2005**, *53,* 5377–5384.

(35) Lytra, G.; Tempere, S.; Le Floch, A.; Revel, G. de; Barbe, J.-C. Study of sensory interactions among red wine fruity esters in a model solution. *J. Agric. Food Chem.* **2013**, *61,* 8504–8513.

(36) Poehlmann, S.; Schieberle, P. Characterization of the aroma signature of styrian pumpkin seed oil (Cucurbita pepo subsp. pepo var. Styriaca) by molecular sensory science. *J. Agric. Food Chem.* **2013**, *61,* 2933–2942.

(37) Ziegleder, G. Linalool contents as characteristic of some flavor grade cocoas. *Z Lebensm Unters Forsch.* **1990**, *191,* 306–309.

(38) Kadow, D. The biochemistry of cocoa flavor - A holistic analysis of its development along the processing chain. *J. Appl. Bot. Food Qual.* **2020**, *93,* 300–312.

(39) Wagner, R. K.; Grosch, W. Key odorants of french fries. *J. Am. Oil Chem. Soc.* **1998**, *75,* 1385–1392.

Figure Captions

Figure 1. Principal component analysis of flavor-active compounds with DoT factor >1 in the reference chocolates

Table 1. Chocolates made from Cocoa of Excellence Reference Liquors that were selected as Reference for the listed Flavor Attributes

sample code	cocoa variety	cocoa bean origin	reference attributes
Ref1	Forastero	Ghana	cocoa, roast degree
Ref2	Criollo	Mexico	fruity (fresh fruit, browned fruit), acidic
Ref3	Trinitario	Dominican Republic	fruity (fresh fruit, browned fruit), acidic
Ref4	Trinitario	Madagascar	fruity (fresh fruit), acidic
Ref5	Nacional / Forastero	Ecuador	floral, astringent, bitter
Ref6	Forastero	Ivory Coast	cocoa, roast degree

Table 2. Odor-Active Compounds perceived during AEDA in at least two Samples and at least with an FD Factor of 16 in one Sample

			retention index on		flavor dilution factor[b]		
no.	compound[a]	odor quality	DB-FFAP	DB-5	Ref4	Ref5	Ref1
1	2- and 3-methylbutanal	malty	875	<700	16	64	64
2	ethyl 2-methylpropanoate[c]	fruity, apple-like	939	746	16	64	1
3	butane-2,3-dione	buttery, caramel	958	<700	64	256	64
4	methyl butanoate[c]	fruity, glue-like	974	<700	16	4	64
5	ethyl butanoate[c]	fruity	1022	803	16	4	4
6	ethyl 2-methylbutanoate[c]	fruity	1035	846	16	64	64
7	ethyl 3-methylbutanoate[c]	fruity	1056	846	16	1	16
8	3-methylbutyl acetate	banana-like, fruity	1113	878	16	1	-
9	unknown	fruity	1259	-	64	4	64
10	oct-1-en-3-one[c]	mushroom-like	1295	976	16	1	-
11	dimethyltrisulfane	cabbage-like	1357	963	16	4	64
12	2,3,5-trimethylpyrazine	earthy, roasty	1391	1000	256	64	64
13	2-methoxy-3-isopropylpyrazine[c]	earthy, green pea-like	1418	1093	256	64	64
14	acetic acid	vinegar-like, pungent	1439	<700	1024	256	1024
15	2-ethyl-3,5-dimethylpyrazine	earthy, roasty	1446	1084	256	64	256
16	2,3-diethyl-5-methylpyrazine	earthy, roasty	1477	1154	256	64	4096
17	2-methoxy-3-sec-butylpyrazine[c]	earthy, green pea-like	1486	1172	16	4	16
18	2-methoxy-3-isobutylpyrazine[c]	green bell pepper-like	1508	1180	256	256	64
19	unknown	fruity, sweaty, pungent	1513	1063	64	4	64
20	linalool	citrus-like, bergamot-like	1536	1099	4	4	16
21	2-methylpropanoic acid	cheesy, sweaty	1555	794	16	4	16
22	(2*E*,6*Z*)-nona-2,6-dienal[c]	cucumber-like, pungent	1567	1159	-	4	16
23	butanoic acid	sweaty, vomit-like, rancid	1617	820	16	64	16
24	phenylacetaldehyde	honey-like, bees wax-like	1629	1039	64	64	64
25	2-methyl-3-(methyldisulfanyl)furan[c]	nutty, meaty, seasoning-like	1650	1170	1024	256	64
26	2- and 3-methylbutanoic acid	sweaty, cheesy	1655	859	256	1024	4096
27	(2*E*,4*E*)-nona-2,4-dienal[c]	cardboard-like, fatty, rancid	1687	1212	16	4	16
28	3-methylnonane-2,4-dione[c]	flowery, fruity, rose-like	1699	-	256	256	64
29	dimethyltetrasulfane[d]	seasoning-like, cabbage-like	1716	1212	256	256	1024
30	unknown	meaty, seasoning-like	1738	-	16	1	4
31	ethyl phenylacetate	flowery, honey-like	1773	1241	4	16	4
32	2-phenylethyl acetate	flowery, dried fruits-like	1799	1257	1024	256	64
33	2-methoxyphenol	gammon-like, smoky	1847	1087	256	256	1024
34	ethyl 3-phenylpropanoate	fruity, cinnamon-like	1868	1347	1024	256	256
35	2-phenylethan-1-ol	flowery, honey-like	1897	1111	1024	256	1024
36	trans-4,5-epoxy-(*E*)-2-decenal[d]	cardboard-like, metallic	1993	1382	64	256	16
37	γ-nonalactone	coconut-like, peach-like	2007	1362	16	16	16
38	4-ethyl-2-methoxyphenol	smoky, clove-like, spicy	2010	1274	-	1	64
39	4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	caramel-like	2016	-	1	1	1
40	4-methylphenol	horse stable-like	2073	1079	16	1	16
41	2-methoxy-4-propylphenol	smoky, clove-like, spicy	2094	1374	1	1	64
42	ethyl cinnamate	fruity, cinnamon-like	2114	1464	1024	1024	1024
43	γ-decalactone[c]	peach-like	2122	1469	-	4	16
44	4-ethylphenol[c]	leather-like, smoky	2155	-	16	16	16
45	3-ethylphenol[c]	horse stable-like, leather-like	2169	-	16	4	1
46	4-ethenyl-2-methoxyphenol	smoky	2184	1326	1	1	16
47	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like	2186	1108	256	256	4096
48	2,6-dimethoxyphenol[c]	gammon-like, smoky	2256	-	1	16	-
49	phenylacetic acid	bees wax-like	2543	1257	1024	256	1024
50	vanillin	vanilla-like	2554	1402	64	16	4

[a]Identification by comparing RIs, odor qualities and mass spectra to those of reference compounds. [b]FD factors were determined on the DB-FFAP column. [c]Tentative identification by comparing RIs and odor qualities with those of reference compounds. [d]No reference compound was available and tentative identification was based on comparing the RI and odor quality with literature data. [e]This compound was not perceived during the AEDA, but by another sniffer during the analysis of the concentrated distillates

Table 3. Concentrations of selected Odorants and Tastants in the six Reference Chocolates as Means of Triplicates (Standard Deviations < 15%)

	concentration(µg/kg for odorants, mg/100 g for tastants)					
	fruity, acidic			floral, astringent	cocoa-like, roasty	
	Ref4	Ref2	Ref3	Ref5	Ref1	Ref6
odorants						
2-methylbutanal	1450	897	1450	1340	2770	2550
3-methylbutanal	4730	3660	6610	3480	11900	9300
phenylacetaldehyde	2390	1600	2840	1830	4760	3030
2-methylbutanoic acid	4730	6970	7090	5920	11000	6320
phenylacetic acid	18200	16500	32700	20600	40600	25900
3-methylbutanoic acid	11500	11600	20500	11200	23700	14400
acetic acid	3310000	2370000	2330000	1750000	1460000	1660000
ethyl 2-methylbutanoate	2.61	3.03	2.00	1.84	1.43	1.73
ethyl 3-methylbutanoate	2.08	3.41	3.75[a]	1.46	2.27	2.06
3-methylbutyl acetate	277	45.6	229	92.6	46.2	35.6
ethyl phenylacetate	289	194	790	270	178	91.8
2-phenylethyl acetate	2010	1610	3220	768	597	496
ethyl cinnamate	402	138	161	116	95.3	68.2
linalool	36.8	21.7	256	124	446	196
2-phenylethan-1-ol	5950	4430	8650	8650	5530	4970
2,3-diethyl-5-methylpyrazine	2.24	0.89	2.25	0.87	8.22	6.55
2,3,5-trimethylpyrazine	699	496	509	119	472	197
2-ethyl-3,5-dimethylpyrazine	109	111	204	29.6	151	61.4
2-methoxyphenol	88.9	171	135	35.5	88.8	19.6
4-methylphenol	40.4	9.54	25.0	10.5	21.0	12.6
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	45.4	36.5	48.5	26.0	45.3	44.2
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	1360	3790	1100	1280	4470	6310
dimethyltrisulfane	13.3	6.44	13.4	4.20	52.8	33.9
2-methyl-3-(methyldisulfanyl)furan	2.70	0.445	0.927	0.328	2.50	1.85
γ-decalactone	23.2	14.2	26.2	37.4	42.9	31.5
γ-nonalactone	112	78.0	308	369	595	107
vanillin	63.0	177	133	153	205	126
tastants						
citric acid[b]	487	469	317	337	442	605
lactic acid[b]	641	242	267	117	140	82.8
theobromine[b]	719	788	1210	1130	1030	922
caffeine[b]	155	216	198	168	121	109
(−)-epicatechin[b]	153	132	105	412	101	117
procyanidin B2[b]	87.8	97.8	63.3	242	66.5	92.8
procyanidin C1[b]	55.3	57.2	44.4	181	41.2	53.0
cyclo(L-pro-L-val)[b,c]	5.59	4.51	6.70	4.73	13.7	5.21

[a]mean of duplicate. [b]concentrations in the whole chocolate calculated from the concentrations analyzed in the defatted chocolates with a fat content of 40%. [c]data were taken from a previous publication.[30]

Table 4. Dose over Threshold Factors of selected Odorants and Tastants in the Reference Chocolates (Different Letters After the Value Indicate a Significant Difference between the Samples for the Compound)

		dose over threshold factor					
	threshold	fruity, acidic			floral, astringent	cocoa-like, roasty	
	value[a]	Ref4	Ref2	Ref3	Ref5	Ref1	Ref6
odorants							
2-methylbutanal	34.0[a]	42.7 c	26.4 d	42.6 c	39.3 c	81.4 a	74.9 b
3-methylbutanal	15.0[a]	316 d	244 de	440 c	232 e	793 a	620 b
phenylacetaldehyde	34.0[a]	70.3 c	47.1 e	83.5 b	53.7 d	140 a	89.2 b
2-methylbutanoic acid	114[a]	41.5 e	61.2 bc	62.2 b	51.9 d	96.8 a	55.4 cd
phenylacetic acid	26.0[a]	701 d	633 d	1260 b	791 cd	1560 a	997 c
3-methylbutanoic acid	11.0[a]	1040 d	1060 d	1870 b	1020 d	2150 a	1310 c
acetic acid	350[b]	9470 a	6770 b	6660 b	5000 c	4180 d	4750 c
ethyl 2-methylbutanoate	0.370[a]	7.05 a	8.18 a	5.40 b	4.98 bc	3.87 c	4.68 b
ethyl 3-methylbutanoate	0.980[a]	2.12 b	3.48 a	3.82 a	1.49 c	2.31 b	2.10 b
3-methylbutyl acetate	76.0[a]	3.64 a	<1 d	3.02 b	1.22 c	<1 d	<1 d
ethyl phenylacetate	300[a]	<1 b	<1 c	2.63 a	<1 b	<1 c	<1 d
2-phenylethyl acetate	14000[a]	<1 b	<1 c	<1 a	<1 d	<1 e	<1 f
ethyl cinnamate	7100[c]	<1 a	<1 c	<1 b	<1 d	<1 e	<1 f
linalool	3.40[a]	10.8 e	6.39 e	75.4 b	36.6 d	131 a	57.6 c
2-phenylethan-1-ol	490[a]	12.1 b	9.03 d	17.6 a	17.7 a	11.3 bc	10.1 cd
2,3-diethyl-5-methylpyrazine	7.20[a]	<1 c	<1 d	<1 c	<1 d	1.14 a	<1 b
2,3,5-trimethylpyrazine	180[a]	3.88 a	2.75 bc	2.83 b	<1 e	2.62 c	1.09 d
2-ethyl-3,5-dimethylpyrazine	1.70[a]	64.3 c	65.5 c	120 a	17.4 e	89.0 b	36.1 d
2-methoxyphenol	1.80[d]	49.4 c	95.2 a	75.0 b	19.7 d	49.3 c	10.9 e
4-methylphenol	3.30[d]	12.2 a	2.89 d	7.58 b	3.17 d	6.35 c	3.81 d
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	0.200[e]	227 ab	182 c	242 a	130 d	227 a	221 b
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	27.0[a]	50.4 d	140 c	40.9 e	47.3 d	165 b	234 a
dimethyltrisulfane	0.030[a]	444 c	215 d	446 c	140 e	1760 a	1130 b
2-methyl-3-(methyldisulfanyl)furan	0.370[a]	7.29 a	1.20 d	2.51 c	<1 d	6.76 a	4.99 b
γ-decalactone	4800[a]	<1 d	<1 e	<1 d	<1 b	<1 a	<1 c
γ-nonalactone	1300[f]	<1 c	<1 c	<1 b	<1 b	<1 a	<1 c
vanillin	140[a]	<1 d	1.27 ab	<1 c	1.10 bc	1.46 a	<1 c
tastants							
acetic acid	2000[g]	27.6 a	19.7 b	19.4 b	14.6 c	12.2 d	13.8 c
citric acid	2600[g]	9.75 b	9.39 bc	6.34 d	6.74 d	8.84 c	12.1 a
lactic acid	15400[g]	4.62 a	1.74 c	1.92 b	<1 e	1.01 d	<1 f
theobromine	800[h]	49.9 d	54.7 d	83.7 a	78.6 a	71.4 b	64.0 c
caffeine	750[h]	10.6 c	14.8 a	13.6 b	11.6 c	8.32 d	7.50 d
(−)-epicatechin	800[i]	6.60 b	5.67 c	4.54 d	17.7 a	4.35 d	5.03 cd
procyanidin B2	200[i]	7.59 b	8.45 b	5.47 c	20.9 a	5.75 c	8.02 b
procyanidin C1	300[i]	2.13 b	2.20 b	1.71 c	6.96 a	1.59 c	2.04 b
cyclo(L-pro-L-val)[j]	1280[h]	<1 c	<1 e	<1 b	<1 de	<1 a	<1 cd

[a]odor threshold value in μg/kg according to reference 29. [b]odor threshold value in μg/kg according to reference 36. [c]odor threshold value in μg/kg according to reference 15. [d]odor threshold value in μg/kg according to reference 17. [e]odor threshold value in μg/kg according to reference 39. [f]odor threshold value in μg/kg according to reference 19. [g–i]taste threshold in μmol/kg for [g]sour, [h]bitter and [i]astringent perception according to reference 26. [j]data were taken from a previous publication.[30]

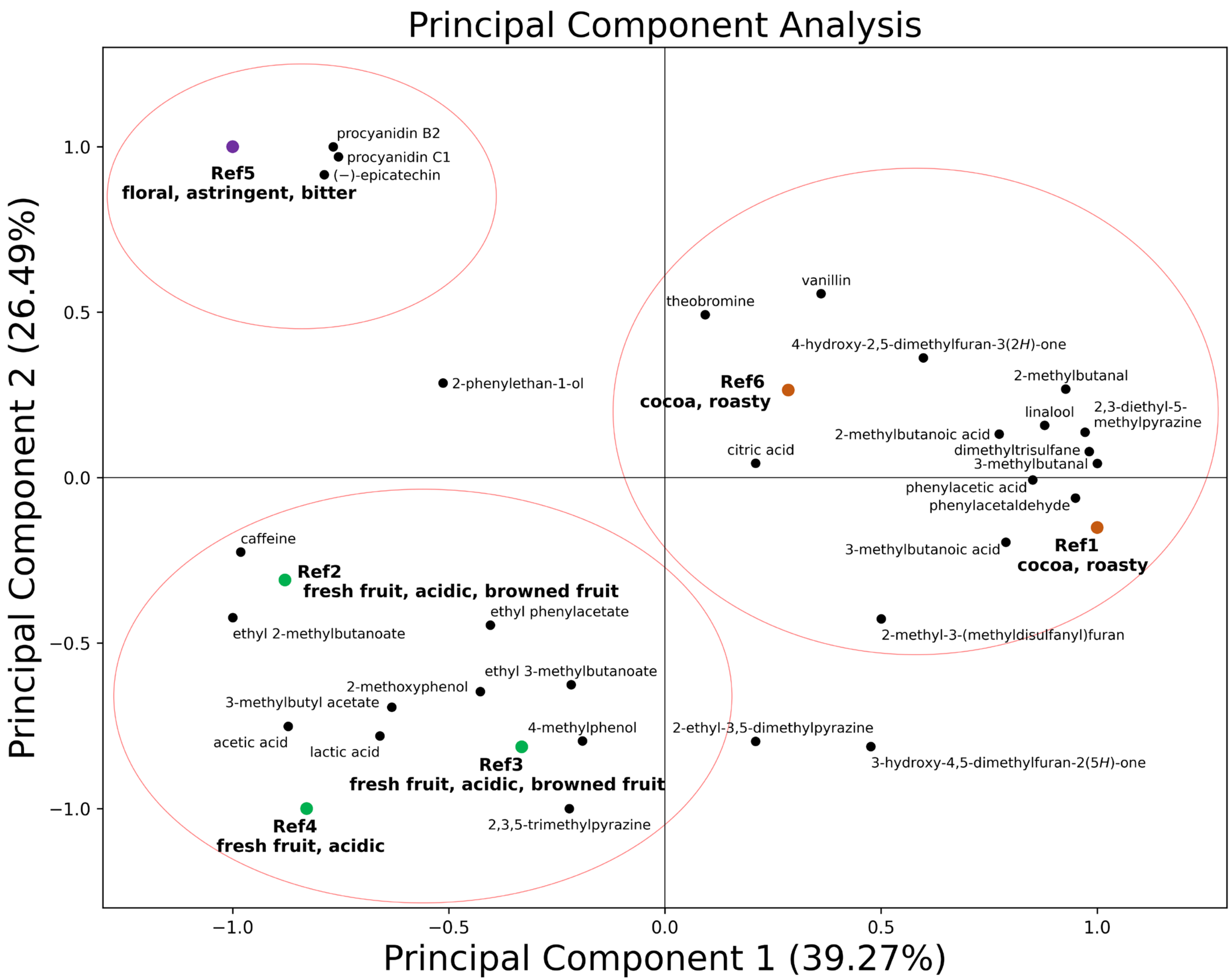

Figure 1. Principal component analysis of flavor-active compounds with DoT factor >1 in the reference chocolates

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