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Flavor-active compounds in cocoa and chocolate – impact of water and variety

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Contents

1 Summary

Chocolate is consumed worldwide for its unique flavor, which mainly forms during fermentation, drying, and roasting of cocoa beans. A novel chocolate making process has been developed that produces chocolates (NPCs) with different flavor profiles than the ones of traditionally processed chocolates (TPCs). Even though the novel process does not involve roasting, both types of chocolates contain similar concentrations of odorants known to be formed mainly during thermal treatments, such as Strecker aldehydes and pyrazines. In the novel process, fermented and dried cocoa beans are treated with water, which has led to the hypothesis that the compounds are released from precursors upon water contact.

To test this hypothesis, we quantitated odor-active compounds in fermented and dried cocoa beans before and after water treatment. Strecker aldehydes showed the highest increase in concentration by water treatment, with a 66-fold increase in 3-(methylsulfanyl)propanal and a 50-fold increase in phenylacetaldehyde. Most other cocoa key odorants also increased in concentration. The release of odorants, such as Strecker aldehydes, by saliva is assumed to highly impact the retronasal odor perception of TPCs. It was previously not clear whether NPCs release similar amounts of odorants as TPCs when treated with water, as the cocoa beans already had faced intense water contact in the novel process. To investigate this, the concentrations of selected odorants before and after water treatment were compared in an NPC and a TPC, both made from the same batch of the already analyzed cocoa beans. Interestingly, the concentrations of nearly all quantitated odorants increased to a higher extent in the NPC than in the TPC upon water treatment. Our results thus also demonstrated that a water-free sample work-up is only adequate to characterize the orthonasal odor profile. Water treatment before sample work-up is essential to represent the retronasal odor perception. Consequently, sample work-up with water was chosen for all further experiments.

The flavor compound composition of cocoa and chocolate has been the subject of numerous studies with the main focus on flavor development along the processing chain. The flavor compound composition of chocolate is not only influenced by processing conditions such as fermentation, drying, and roasting parameters but also by the cocoa bean itself. Cocoa products of defined variety and origin are described in the literature with certain fine flavor attributes. However, most studies focusing on sensory-active compounds were carried out with cocoa products of undefined variety and origin. Consequently, the molecular background of fine flavor properties was previously not well understood. To decode selected fine flavor properties on a molecular level, flavor-active compounds were analyzed in six sensory reference chocolates. The chocolates showed distinct flavor properties and were referenced either as fruity and acidic, cocoa-like and roasty, or floral and astringent. The aroma extract dilution analysis (AEDA) of three chocolates revealed that the fine flavor properties were evoked by certain concentrations of already known cocoa and chocolate key odorants. In the next step, selected odorants and tastants were quantitated in all six chocolates, and dose over threshold (DoT) factors were calculated. Acidic and fruity flavor notes were associated with high DoT factors of acetic acid and fruity smelling esters, such as ethyl 2-methylbutanaote, ethyl 3-methylbutanoate, and 3-methylbutyl acetate. Cocoa-like and roasty flavor notes were associated with high DoT factors of 2-methylbutanal, 3-methylbutanal, 4-hydroxy-2,5-

dimethylfuran-3(2*H*)-one, and dimethyltrisulfane. Finally, floral and astringent flavor notes were linked to high DoT factors of (–)-epicatechin, procyanidin B2, procyanidin C1, and 2-phenylethan-1-ol.

Fine flavor attributes such as the ones decoded in the second part of this thesis are mainly described in single variety cocoa products. The cocoa bean variety is a criterion for classifying cocoa as fine flavor cocoa, even though the impact of the variety on the flavor compound composition of cocoa and chocolate has not yet been studied comprehensively. To fill this gap, flavor-active compounds were analyzed in dark chocolates made from different cocoa bean varieties, namely three Forastero chocolates, six Trinitario chocolates, six Criollo chocolates, and one Nacional chocolate. The DoT factors were normalized to the cocoa content and compared. The three Forastero chocolates were similar in their flavor compound composition and were characterized by high DoT factors of 3-methylbutanal, dimethyltrisulfane, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 2-methylbutanoic acid, 3-methylbutanoic acid, phenylacetic acid, and linalool. The flavor compound profiles of the Criollo and Trinitario chocolates showed greater variations, indicating that the variety is not the only determinant of the flavor compound composition of dark chocolates. Three Trinitario chocolates and a Criollo chocolate showed especially high DoT factors of fruity smelling esters and acetic acid, while some other Trinitario and Criollo chocolates showed similarities with the Forastero chocolates. Finally, the flavor compound compositions of the single variety dark chocolates could be at least partly linked to the cocoa bean variety.

2 Zusammenfassung

Schokolade wird weltweit wegen ihres einzigartigen Aromas konsumiert, welches sich hauptsächlich bei der Fermentation, Trocknung und Röstung von Kakaobohnen bildet. Schokoladen, welche mit einem neuartigen Produktionsverfahren hergestellt wurden (NPCs), besitzen andere Flavor-Profile als die Schokoladen herkömmlicher Verarbeitung (TPCs). Obwohl bei dem neuen Verfahren nicht geröstet wird, enthalten beide Arten von Schokolade ähnliche Konzentrationen an Geruchsstoffen wie z. B. Strecker-Aldehyde und Pyrazine, die laut Literatur hauptsächlich bei thermischen Behandlungen gebildet werden. Im neuen Herstellungsprozess werden die fermentierten und getrockneten Kakaobohnen mit Wasser behandelt, was zu der Hypothese geführt hat, dass die Verbindungen bei Wasserkontakt aus Präkursoren freigesetzt werden.

Um diese Hypothese zu prüfen, haben wir geruchsaktive Verbindungen in fermentierten und getrockneten Kakaobohnen vor und nach Wasserbehandlung quantifiziert. Strecker-Aldehyde zeigten den höchsten Konzentrationsanstieg durch die Wasserbehandlung, mit einem 66-fachen Anstieg von 3(Methylsulfanyl)propanal und einem 50-fachen Anstieg von Phenylacetaldehyd. Auch die meisten anderen Schlüsselgeruchsstoffe nahmen in ihrer Konzentration zu. Es wird angenommen, dass die Freisetzung von Geruchsstoffen, wie Strecker-Aldehyden, durch Speichel die retronasale Geruchswahrnehmung von TPCs stark beeinflusst. Bisher war nicht klar, ob NPCs bei der Behandlung mit Wasser ähnliche Mengen an Geruchsstoffen freisetzen wie TPCs, da die Kakaobohnen bei dem neuen Verfahren bereits intensiv mit Wasser in Berührung gekommen waren. Um dies zu untersuchen, wurden die Konzentrationen ausgewählter Geruchsstoffe vor und nach Wasserbehandlung in einer NPC und einer TPC verglichen. Beide wurden aus der gleichen Charge der bereits analysierten Kakaobohnen hergestellt. Interessanterweise stiegen die Konzentrationen fast aller quantifizierten Geruchsstoffe nach der Wasserbehandlung in der NPC stärker an als in der TPC. Unsere Ergebnisse zeigten somit weiterhin, dass eine wasserfreie Probenaufbereitung nur geeignet ist, um das orthonasale Geruchsprofil zu charakterisieren. Um die retronasale Geruchswahrnehmung darzustellen, ist dagegen eine Wasserbehandlung vor der Probenaufbereitung unerlässlich. Folglich wurde für alle weiteren Experimente eine Probenaufbereitung mit Wasser gewählt.

Die flavor-aktiven Verbindungen von Kakao und Schokolade wurden in zahlreichen Studien untersucht, wobei der Schwerpunkt auf der Flavor-Entwicklung während der Verarbeitung lag. Die flavor-aktiven Verbindungen der Schokolade werden nicht nur durch Verarbeitungsbedingungen wie Fermentations-, Trocknungs- und Röstparameter beeinflusst, sondern auch durch die Kakaobohne selbst. Kakaoprodukte definierter Sorten und Herkunft werden in der Literatur mit bestimmten Fine-Flavor-Eigenschaften assoziiert. Die meisten Studien, die sich auf sensorisch aktive Verbindungen konzentrierten, wurden jedoch mit Kakaoprodukten undefinierter Sorte und Herkunft durchgeführt. Folglich war der molekulare Hintergrund von Fine-Flavor-Eigenschaften bisher nicht gut verstanden. Um ausgewählte Fine-Flavor-Eigenschaften auf molekularer Ebene zu entschlüsseln, wurden flavor-aktive Verbindungen in sechs sensorischen Referenzschokoladen analysiert. Die Schokoladen zeigten ausgeprägte Flavor-Eigenschaften und waren Referenzen entweder für fruchtig und sauer, kakaoartig und röstig, oder blumig und adstringierend. Die Aromaextrakt-Verdünnungsanalyse (AEDA) von

drei Schokoladen zeigte, dass die Fine-Flavor-Eigenschaften durch bestimmte Konzentrationen bereits bekannter Kakao- und Schokoladenschlüsselgeruchsstoffe hervorgerufen wurden. Im nächsten Schritt wurden ausgewählte Geruchs- und Geschmacksstoffe in allen sechs Schokoladen quantifiziert und Dose-over-Threshold (DoT)-Faktoren berechnet. Saure und fruchtige Noten wurden mit hohen DoT-Faktoren von Essigsäure und fruchtig riechenden Estern wie Ethyl-2-methylbutanoat, Ethyl-3-methylbutanoat und 3-Methylbutylacetat in Verbindung gebracht. Kakaoartige und röstige Noten waren mit hohen DoT-Faktoren von 2-Methylbutanal, 3-Methylbutanal, 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-on und Dimethyltrisulfan assoziiert. Dagegen wurden blumige und adstringierende Noten mit hohen DoT-Faktoren von (–)-Epicatechin, Procyanidin B2, Procyanidin C1 und 2-Phenylethan-1-ol in Verbindung gebracht.

Fine-Flavor-Eigenschaften, wie sie im zweiten Teil dieser Arbeit entschlüsselt wurden, werden hauptsächlich bei sortenreinen Kakaoprodukten beschrieben. Die Kakaobohnensorte ist ein Kriterium für die Einstufung von Kakao als Fine-Flavor-Kakao, obwohl der Einfluss der Sorte auf die flavor-aktiven Verbindungen in Kakao und Schokolade noch nicht umfassend untersucht worden ist. Um diese Lücke zu schließen, wurden flavor-aktive Verbindungen in dunklen Schokoladen verschiedener Kakaobohnensorten analysiert, nämlich in drei Forastero-Schokoladen, sechs Trinitario-Schokoladen, sechs Criollo-Schokoladen und einer Nacional-Schokolade. Die DoT-Faktoren wurden auf den Kakaogehalt normiert und verglichen. Die drei Forastero-Schokoladen waren ähnlich in ihren Flavor-Verbindungen und zeichneten sich durch hohe DoT-Faktoren von 3-Methylbutanal, Dimethyltrisulfan, 4-Hydroxy-2,5-dimethylfuran-3(2*H*)-on, 2-Methylbuttersäure, 3-Methylbuttersäure, Phenylessigsäure und Linalool aus. Die flavor-aktiven Verbindungen von Criollo- und Trinitario-Schokoladen wiesen größere Unterschiede auf, was darauf hindeutete, dass die Sorte nicht der einzige bestimmende Faktor für die flavor-aktiven Verbindungen von dunklen Schokoladen ist. Drei Trinitario-Schokoladen und eine Criollo-Schokolade wiesen besonders hohe DoT-Faktoren von fruchtig riechenden Estern und Essigsäure auf, während einige andere Trinitario- und Criollo-Schokoladen Ähnlichkeiten mit den Forastero-Schokoladen aufwiesen. Schlussendlich konnten die flavor-aktiven Verbindungen in den sortenreinen dunklen Schokoladen zumindest teilweise auf die Kakaobohnensorte zurückgeführt werden.

3 Abbreviations and nomenclature

Abbreviations

AEDA	aroma extract dilution analysis
cAMP	cyclic adenosine monophosphate
CoEx	Cocoa of Excellence
DoT	dose over threshold
FD	flavor dilution
GC-MS	gas chromatography-mass spectrometry
GC-O	gas chromatography-olfactometry
GTP	guanosine 5'-triphosphate
HPLC-MS/MS	high performance liquid chromatography-mass spectrometer/mass spectrometer
HVD	high vacuum distillation
NPC	chocolate made with the novel processing technology
OAV	odor activity value
ORC	olfactory receptor cell
OTV	odor threshold value
PC	principal component
PCA	principal component analysis
RI	retention index
SAFE	solvent assisted flavor evaporation
SDE	simultaneous steam distillation/extraction
TPC	chocolate made with the traditional processing technology
WT	water treatment

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
δ-decenolactone	6-pentyl-5,6-dihydro-2*H*-pyran-2-one
(–)-epicatechin	(2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3,5,7-triol
trans-4,5-epoxy-(*E*)-2-decenal	(2*E*)-3-(3-pentyloxiran-2-yl)prop-2-enal
ethyl cinnamate	ethyl (2*E*)-3-phenylprop-2-enoate
2-isobutyl-3-methoxypyrazine	2-methoxy-3-(2-methylpropyl)-pyrazine
lactic acid	2-hydroxypropanoic acid
linalool	3,7-dimethylocta-1,6-dien-3-ol
2-methoxy-3-isopropylpyrazine	2-methoxy-3-(propan-2-yl)pyrazine
2-methoxy-3-sec-butylpyrazine	2-(butan-2-yl)-3-methoxypyrazine
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

4 Introduction

4.1 Molecular sensory science

4.1.1 Odor-active compounds and odor perception

The sensory properties of a food are important for consumer acceptance. Odor, as part of the flavor, is the most important sensation during food consumption.[1] In contrast to gustation, the olfactory system can distinguish a wide range of sensations.[2–5] Odors can be perceived either orthonasally or retronasally, depending on the pathway from the food to the olfactory epithelium in the nasal cavity.[1,6] In orthonasal odor perception, odorants from the food evaporate into the surrounding air and are inhaled through the nose into the nasal cavity.[1] In retronasal odor perception, odorants enter the nasal cavity via the mouth and the oropharyngeal pathway during eating.[1,7]

All odor-active compounds are volatile but not all volatile compounds present in foods are odor-active.[1] In fact, only 3% of all identified food volatiles were shown to contribute to the olfactory properties of foods.[2] Odor-active compounds have a relatively low molecular mass, sufficient hydrophobicity, and the ability to bind to at least one olfactory receptor protein type in the olfactory epithelium.[1,8] The latter is normally achieved through specific functional groups in combination with a hydrophobic part of the molecule.[1] Interestingly, odor quality cannot be predicted by the chemical structure of an odorant, and compounds with similar chemical structures can evoke completely different odors.[3] Finally, to be odor-active, a compound must be present in an amount that exceeds its specific odor threshold concentration to be able to activate the olfactory system.[1,2] Odor threshold concentrations vary drastically for different odorants and also differ between orthonasal and retronasal perception.[1,2,6]

The olfactory transduction pathway starts when odorants reach the olfactory epithelium and bind to the receptors of olfactory receptor cells (ORCs).[9] The biological structures involved in the process of odor perception are shown in Figure 1. About 10 million ORCs are located in the human olfactory epithelium.[1,5] ORCs are bipolar sensory neurons and have a dendrite with an olfactory knob that contains tiny cilia on one side. The cilia are embedded in mucus and exposed to the exterior of the tissue, where odorants can bind to receptors.[5,9] On the rear side, ORCs are connected to the olfactory bulb in the brain via axons.[5] It is assumed that odorant-binding proteins transport the hydrophobic odorants through the aqueous mucus of the olfactory epithelium to the olfactory receptors in the membrane of the ORCs.[5,8] There are several types of receptor classes and about 400 different olfactory receptor proteins exist.[1,5,9,10] One ORC contains only one type of protein.[1,9] Each type of receptor can interact with several odorants and each odorant may activate multiple receptor proteins.[3,5,11]

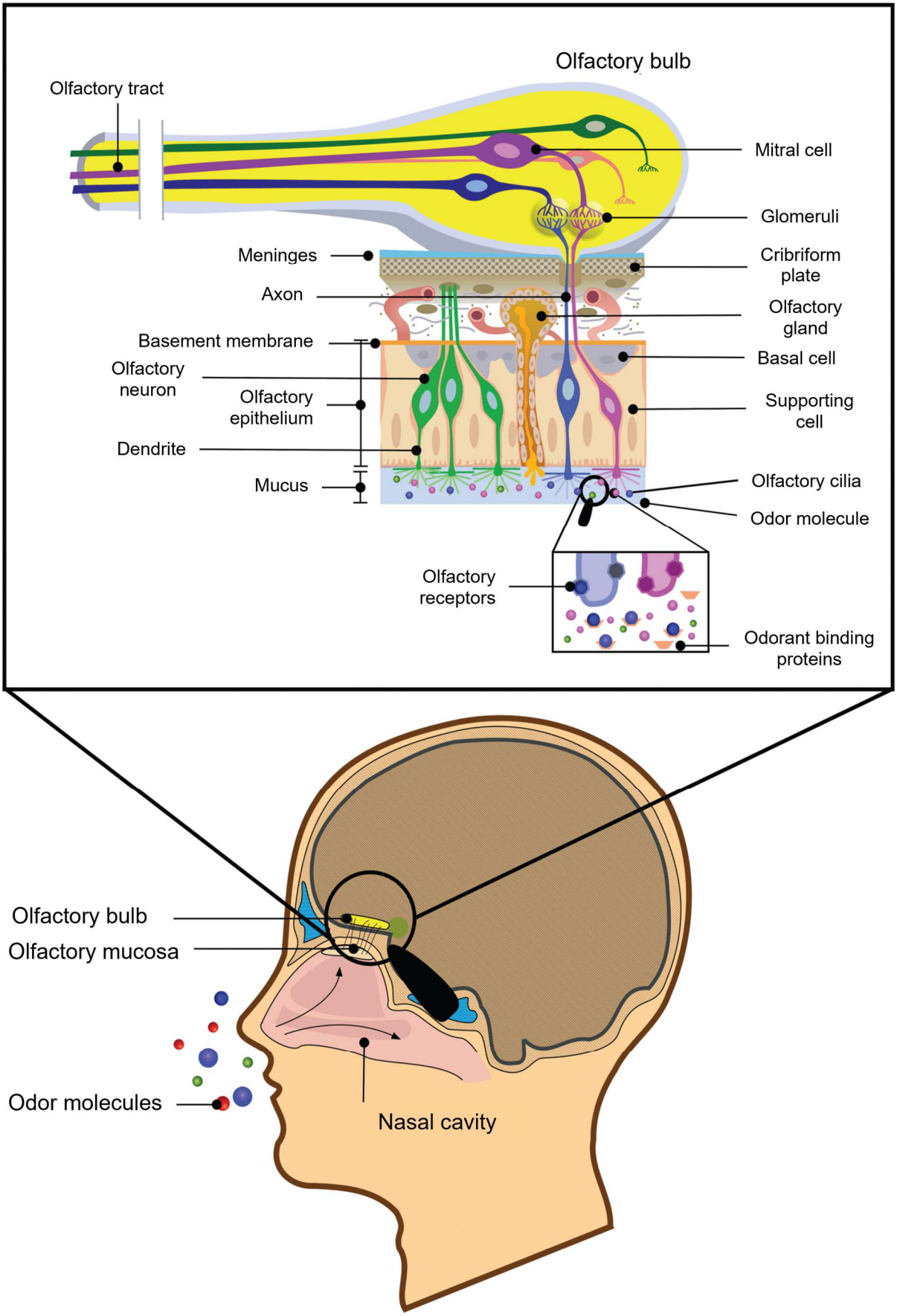

Figure 1: Structures involved in odor perception[5]

The binding of a sufficient number of odor-active molecules to olfactory receptors in the membrane of an ORC initiates an olfactory transduction cascade (Figure 2).[1,8,9] The receptor is coupled to a G protein consisting of the three subunits α, β, and γ, and is located inside the cell.[9,11] When an odorant binds to a receptor, the associated G protein exchanges bound guanosine 5'-diphosphate for guanosine 5'-triphosphate (GTP). GTP dissociates the α subunit from the G protein, while β and γ remain. Dissociation of the α subunit activates the enzyme adenylate cyclase in the membrane. Adenosine triphosphate is converted into cyclic adenosine monophosphate (cAMP).[9] The secondary messenger cAMP activates cyclic nucleotide-gated ion channels in the ciliary membrane.[9,11] The opening of the channels leads to an influx of sodium, potassium, and calcium ions. Incoming calcium ions further activate chloride channels, which lead to an efflux of chloride ions.[9] The generated ion flux leads to depolarization of the cell and converts the chemical signal into an electrical one.[1,8,9,11] The electrical signal is transmitted via axons to the olfactory bulb in the brain.[1,9] Axons emerging from ORCs with the same receptor type converge in the same glomeruli in the olfactory bulb.[1,10,12–14] The spatial organization of the glomeruli results in the projection of the activated ORCs in a topographic map.[12,13] This specific, spatial activation pattern is recognized by the brain for a particular odor.[1,12,13] Different odorants evoke different combinations of ORCs and ORCs can detect even small changes in odorant concentrations.[5,9]

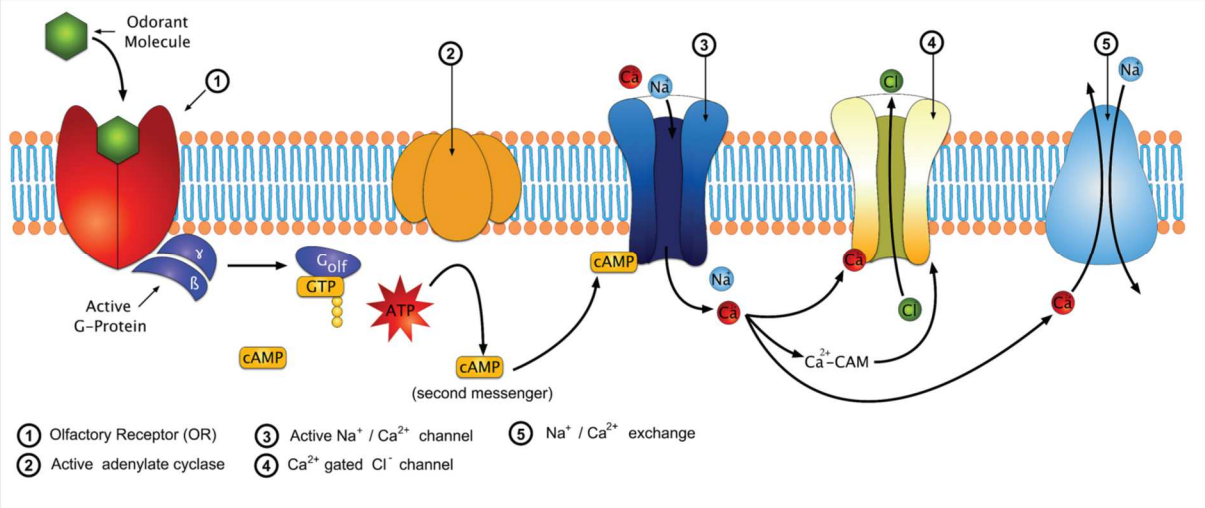

Figure 2: Olfactory signal transduction cascade in the olfactory receptor cell[5]

4.1.2 Analysis of odor-active compounds

In the past, it was often assumed that all volatiles of a food somehow contribute to its odor.[15] Nowadays, it is known that only a small part of volatiles contribute to the odor of food. Odor-active compounds that contribute to the overall odor of food are called key odorants. Key odorants and their contribution to the overall odor can be identified by the approach described in this section.[2]

First, volatiles are isolated from the food and the volatile fraction is screened for odor-active compounds by gas chromatography-olfactometry (GC-O). Volatiles are extracted from a homogenized sample by using a non-polar, organic solvent with a low boiling point. Non-volatiles are subsequently removed from the extract, most commonly by solvent assisted flavor

evaporation (SAFE). Non-volatiles disturb the separation of volatiles in gas chromatographic analysis and negatively impact the performance of the analytical column in the long term.[1] In contrast to simultaneous extraction of steam distillates by solvents (SDE), SAFE is carried out at mild temperatures below 40 °C, which reduces the risk of artifact formation.[16] High temperatures must be avoided throughout the whole sample work-up, as the formation of odor-active artifacts can substantially change the original odorant composition of the food.[1] Another advantage of SAFE is that polar volatiles and volatiles with higher boiling points are less discriminated and transferred to the distillate with higher recovery yields than in older high vacuum transfer (HVT) approaches.[1,16] In addition, SAFE is by far less time-consuming.[16] After SAFE, the distillate is concentrated. Concentration may result in the loss of highly volatile, odor-active compounds that are consequently not detected in a GC-O analysis of the concentrate. If these compounds are of interest, they can be screened separately with headspace methods.[1]

An aliquot of the SAFE concentrate containing the extracted volatiles is injected into the GC-O for analysis (Figure 3).[1] GC-O differentiates odor-active compounds from odorless volatiles. Typically, cold on-column injectors are used in combination with a low GC oven start temperature to reduce the thermal impact on the odorants during analysis. The volatiles are separated on the analytical column, and at the end of the column, the effluent is split into two streams.[1] One stream is transferred to a conventional detector such as a flame ionization detector (FID), or a mass spectrometer (MS). The other stream is transferred to a “sniffing port” where a person constantly sniffs the outcoming gas stream. The “sniffer” notes the retention time and odor quality of each perceived odor event.[1,17] The sniffer should be a trained person who can quickly recall odor descriptions from a set of vocabulary that has been learned with reference substances of unequivocally defined odors. GC-O analysis should be performed by at least two sniffers with different anosmia, and the results should be combined. Finally, a table with odor qualities and retention times is obtained. Optimally, the distillate for GC-O screening yields a table with 30 to 60 odor-active compounds. The retention times are converted into retention indices (RIs) to obtain data that is less dependent on the instrument parameters and thus, better comparable with literature data.[1]

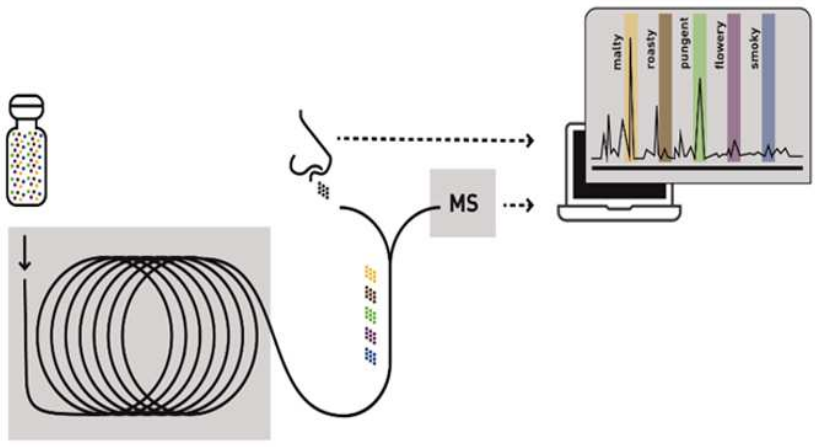

Figure 3: Principle of a GC-O[17]

Odor-active compounds are usually ranked before identification to concentrate the effort on the most odor-potent compounds. The ranking can be done either based on the odor intensity perceived at the sniffing port or based on the detection frequency if the concentrate is analyzed by numerous panelists. However, none of these two ranking methods provide a sufficient differentiation of the odor potencies of very intense odors. A better ranking of odor potencies, especially of compounds with high odor potencies, is achieved by dilution to threshold methods.[1] If the volatiles are present in organic solvents, an aroma extract dilution analysis (AEDA) is applied.[1,18] In practice, the concentrate is diluted stepwise to reduce the injected amount by a factor of 2 or 3 at each dilution. Dilution continues as long as odor-active compounds are perceived in the GC-O analysis. Finally, AEDA provides a flavor dilution (FD) factor for each odor-active compound. The FD factor corresponds to the highest dilution at which the compound was detected during GC-O analysis.[1]

The identification of odor-active compounds is based on odor quality, RI, and mass spectrum. First, the odor quality and RI are compared with literature and reference compounds analyzed with the same device. The analysis of the concentrate and the reference compounds on another stationary phase with a different polarity further confirms the identity. The mass spectrum is the final confirmation or offers further possibilities if the comparison with literature data and reference compounds was not successful.[1] GC-O analysis identifies odor-active compounds within volatiles, but the importance of the odorants for the overall odor cannot unequivocally be assessed. Losses of compounds with very high and very low boiling points during sample work-up change the original odorant profile. Further, the volatility of a compound is not reflected in the GC-O analysis, and all compounds are perceived individually. During food consumption, the odorants are perceived as a mixture with suppressive, additive, and synthetic effects playing a role.[1] Therefore, sensory experiments based on exact quantitative data are essential.[1,15]

Quantitation is only precise when a stable isotope dilution assay (SIDA) is used.[15,19] A stable isotopically substituted analog of the target compound serves as an internal standard.[1,15,19] The isotopically substituted odorants are added at the beginning of the sample work-up and equilibrium between the target compound and the internal standard is being awaited.[1,19] Due to virtually identical physical and chemical properties, the losses of standards and the corresponding target compounds during sample work-up are equal.[1,15,19] Consequently, the concentration ratio of analyte and standard remains constant during sample work, and the internal standards compensate for all odorant losses. The SAFE concentrate containing analytes and standards is then analyzed by gas chromatography-mass spectrometry (GC-MS).[1,19] The concentration of an odorant is calculated from (i) the area ratio of analyte and standard, (ii) the amount of standard added at the beginning of the sample work-up, and (iii) the amount of food used for extraction, by using (iv) calibration lines obtained by analyzing mixtures of analyte and standard of known concentration ratios with the same instrument.[1] To further assess the potency of the individual odorants, the concentrations are related to the individual odor threshold values (OTVs). Usually, the ratio of concentration to odor threshold is simply calculated.[1,20] Nowadays, this ratio is commonly referred to as the odor activity value (OAV).[1] OTVs must be determined in a matrix similar to that of the analyzed food.[1,15] OAVs

are factors for exceeding the OTV and an approximation of the relevance of an odorant to the overall odor of a food.[1]

Nevertheless, OAVs do not consider interactions between odorants in a mixture. Therefore, reconstitution models need to be sensorily evaluated and compared to the original food. A successful reconstitution confirms that all odor-relevant compounds have been correctly identified and quantitated.[1] The odor reconstitution model contains all odorants with OAVs >1 in the calculated concentrations in a matrix that mimics the original food matrix.[1,15] Sensory evaluation of defined odor descriptors by trained panelists reveals whether the model evokes the same odor as the original food. If not, additional odorants must be included in the quantitation.[1] Finally, the contribution of individual odorants to the overall odor of the food is further specified in omission experiments. Only odorants that show an odor difference from the whole model when omitted are considered key odorants.[1] Interestingly, odorants with high OAVs often, but not necessarily, contribute to the odor. In some cases, despite high OAVs, they are suppressed by other odorants.[15] The OAVs of individual odorants vary over a huge range.[2]

4.2 Cocoa and chocolate

4.2.1 Cocoa (*Theobroma cacao*)

Cocoa beans are the main ingredients of chocolate and are responsible for its unique flavor.[21] Cocoa beans are the seeds from cocoa fruits, which grow on cocoa trees (*Theobroma cacao*). *Theobroma cacao* belongs to the subfamily Sterculioidea within the mallow family (Malvaceae). The *Theobroma* genus includes 22 species, but *Theobroma cacao* is the only one cultivated outside its native distribution area. The cocoa tree can reach a height of up to 15 m, but maximum heights of 4–8 m are common. Nowadays, cocoa is grown worldwide within a latitude range of 10° north and south of the equator on both, small and large plantations.[22] The cocoa tree is native to South America and was first domesticated by Mayas and Aztecs.[21,22] Cultivation spread northwards to Mexico and the Caribbean Islands. In the 16th century, Spanish conquistadors brought cocoa to Europe. This was the basis for the later cultivation of cocoa in West Africa, the region with the highest cocoa exports in the world today.[22]

The cocoa tree requires specific environmental conditions.[21,22] The optimum temperature range is between 18 and 32 °C. Rainfall has a major influence on yield, and high amounts of rain distributed throughout the year are essential, preferably 1500–2500 mm per year. The soil must have both, good water retention and good draining with coarse particles to develop a good root system. A pH of 5.0 to 7.5 and other chemical properties such as appropriate amounts of nitrogen and phosphorus are important for growth. Cocoa trees are grown between other food plants such as banana trees to protect them from sunlight and strong winds during the first, fragile growth stages.[22] The flowers emerge from flower cushions directly at the stem of the cocoa tree or at old branches. They develop into young pods after pollination. The pods are fully developed and ready for harvest after approximately 150 days of growth, maturation, and ripening. The stage of ripening is usually evaluated from the pod color which changes from green or purple to various shades of red, orange, or yellow, depending on the genotype. The fruits are 15–25 cm long and are opened after harvest (Figure 4). Each pod contains between 30 and 50 cocoa seeds surrounded by a mucilaginous pulp.[21–23]

Figure 4: Ripe cocoa pods on the tree (left) and an opened cocoa pod (right)[21]

The cocoa bean consists of the shell and the embryo with two cotyledons.[21,23] The cotyledon contains about 80% lipid and protein storage cells and about 20% polyphenol storage cells.[23] Thus, it primarily comprises fat (53–58% of dry weight) and proteins (10–16% of dry weight) with carbohydrates, polyphenols, and alkaloids as other important compounds.[22–24]
Different cocoa varieties are cultivated worldwide, as illustrated in Figure 5. The main cultivation area is West Africa, with Côte d'Ivoire and Ghana producing the largest quantities. Another important region is South America, with Ecuador producing the third-largest share of cocoa in the world.[25] In the world trade market, a distinction is traditionally made between the cocoa genotypes Forastero, Criollo, and Trinitario.[26] Forastero is the most cultivated variety and is characterized by high yields and low susceptibility to plant diseases. Forastero originates from the Amazon region and is nowadays mostly cultivated in West Africa.[22] The fresh beans show a purple color after cutting and develop strong cocoa notes after fermentation and roasting.[21] Forastero beans are often used in cocoa blends for chocolate production.[22] Criollo trees produce lower yields than Forastero trees and are more susceptible to diseases.[21,22] The beans are characterized by their white to light brown color after cutting.[21] Trinitario is considered the result of hybridization between Forastero and Criollo. Another cocoa variety is Nacional, which is grown only in Ecuador and known for its fine flavor characteristics, particularly floral notes.[21,22] Although the traditional classification is widely used, the long history of breeding and hybridization has led to a huge genetic diversity. Recent studies suggest a new classification into about 10 genetic clusters.[27,28] In these studies, only Criollo is maintained as a separate genetic group. Nacional was defined as a separate genetic group by Motamayor et al.,[27] but was grouped with Contamana by Thomas et al.[28]

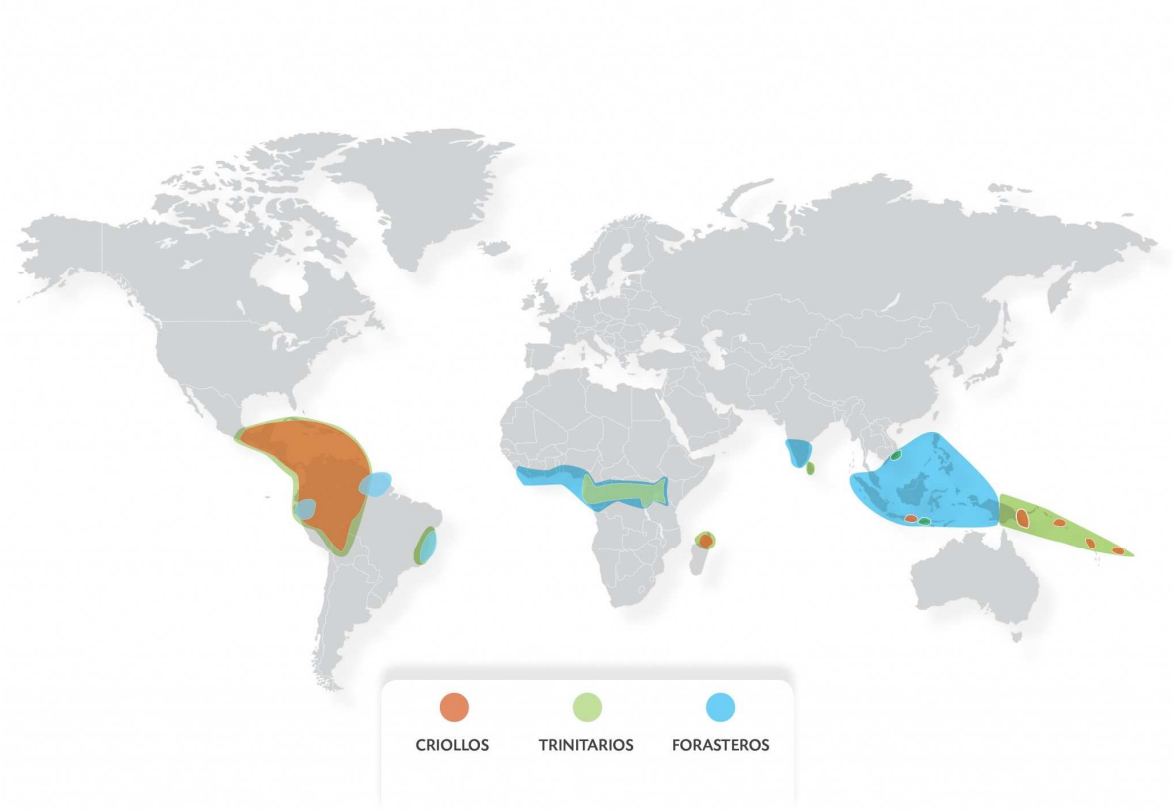

Figure 5: Cultivation areas of the different cocoa bean varieties differentiated on trade level[26]

Besides variety, cocoa is classified as “fine flavor” or “bulk” cocoa on the world trade market, depending on its quality.[29,30] Most of the world’s production is classified as bulk cocoa and used to manufacture cocoa products such as milk and dark chocolate, cocoa mass, cocoa butter, and cocoa powder.[22,29] Furthermore, blends are usually made from bulk cocoa beans. Fine flavor cocoa beans are sold at a higher price, traded in smaller quantities, mainly used to manufacture dark chocolates, and often make a specific contribution to the overall flavor of chocolates.[21,22] The identity of the lot is preserved in the supply chain and the beans are usually from a certain origin and/or variety and/or are harvested in a special way.[21] The share of fine flavor cocoa has increased rapidly in recent years, from less than 5% in 2017[21] to about 12% nowadays.[29] 90% of the fine flavor cocoa comes from Latin America, with Ecuador, the Dominican Republic, and Peru being the three main fine flavor cocoa exporters. The main reason for the increasing production of fine flavor cocoa is the higher demand for high-quality bean-to-bar products by consumers. The most important difference between fine flavor cocoa and bulk cocoa is the flavor quality. Fine flavors include fruity, floral, herbal, woody, nutty, caramel, and chocolate base notes. Nevertheless, classification is usually based on other factors. Besides genetic variety, these include plant morphology, chemical characteristics of the cocoa beans, contamination with insects and molds, presence of off-flavors, and processing parameters of fermentation and drying.[29] Since some of these indicators can be considered subjective and do not objectively reflect flavor quality, the definition of fine flavor cocoa is still controversial.[29,30] Objective indicators that discriminate bulk cocoa from fine flavor cocoa have not yet been clarified.[29]

4.2.2 Cocoa bean processing

Ripe and healthy cocoa pods are harvested in one or two main harvesting periods per year. The pods are opened, and beans and pulp are removed for subsequent fermentation. The pods are opened either immediately after harvesting or after a few days of pod storage.[22] The beans and the adhering pulp are placed in heaps, boxes, or baskets. Having been exposed to various microorganisms from the moment of pod opening, the beans undergo a spontaneous fermentation.[21,22,31,32]

With its high sugar concentration, cocoa pulp is a good fermentation medium for various microorganisms.[22,32] The concentrations of the main substrates and main products of the active microorganisms during fermentation are shown in Figure 6. During the first phase of 24 to 26 hours of fermentation, anaerobic conditions lead to the metabolization of sugars in the pulp.[23,24,31] The acidic conditions favor yeast growth and alcoholic fermentation is dominant during the first two days.[21,22,24,31,32] The pulp is liquefied by the pectinolytic activity of yeasts and bacteria and drains off.[21–24,32] With more pulp draining off and a decreasing sugar breakdown, more air is absorbed.[24] After 48 to 96 hours, the pulp has decomposed and the beans come into direct contact with air.[24,31] Citric acid is metabolized by the yeasts, which leads to an increase in pH and, along with aeration and an increasing alcohol concentration, finally inhibits yeast activity.[32] The aerobic phase is dominated by oxidation and condensation reactions such as the oxidation of ethanol to acetic acid by acetic acid bacteria.[21,24,31] The exothermic processes heat the cocoa beans to 45 to 52 °C.[21,31] The high temperature and the high concentrations of alcohol and acetic acid cause the death of the embryo and inhibit

germination.[21,22,24] Both, acetic acid production and temperature decrease after 4–5 days of fermentation, and lactic acid is formed by lactic acid bacteria.[21,24] The decompartmentalization of enzymes and substrates leads to the formation of important cocoa flavor precursors from carbohydrates and proteins.[21–23,31] The formation of odorants and their precursors are described in section 4.2.4. Furthermore, soluble polyphenols are oxidized and polymerized, leading to a color change from purple to brown.[23,24,31]

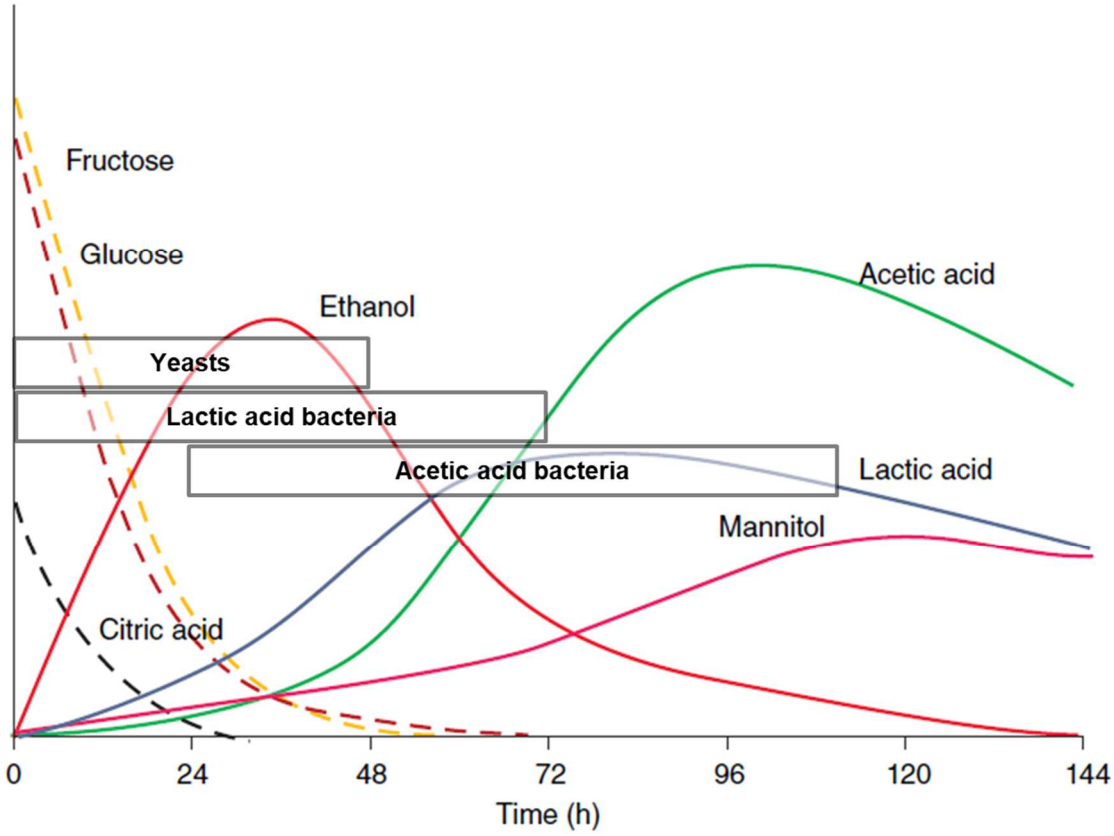

Figure 6: Overview of the activity of microorganisms with the concentrations of their main metabolites during fermentation (modified from Afoakwa[22] and Schwan and Wheals[32])

Fermentation time varies depending on the variety. Criollo beans are usually fermented for one to three days, whereas Forastero beans are fermented for a longer period, usually between five and eight days.[22,24,33] After fermentation, the beans are immediately dried to prevent overfermentation and mold growth during transport and storage.[21,31] Depending on the weather, the beans are sun-dried or artificially dried by hot air produced with wood or oil burners. It takes about one week to reach a moisture content as low as 7–8%.[21,24] During drying, oxidation and polymerization reactions started during fermentation continue.[23]

4.2.3 Chocolate production

Fermented and dried cocoa beans are shipped to the country of chocolate production.[34] The quality of cocoa beans is crucial for the quality of chocolate and is therefore evaluated before cocoa beans are processed. Important quality parameters include degree of fermentation, fat

content, color, flavor quality, and defects such as broken and infested beans.[22] The process of traditional chocolate production is shown in Figure 7.

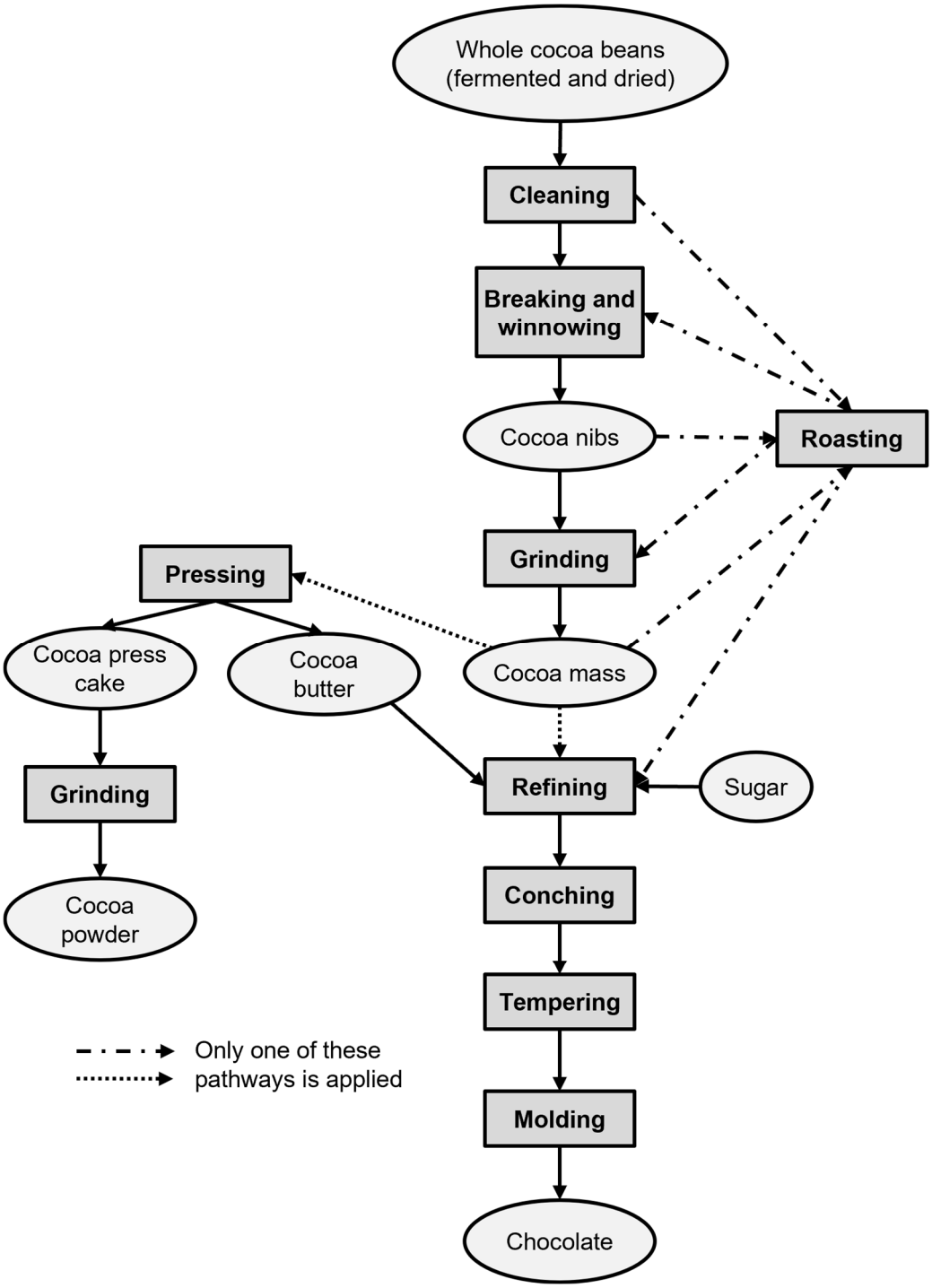

Figure 7: Simplified illustration of traditional chocolate processing

First, cocoa beans are cleaned,[34,35] and extraneous material is removed by sieving.[22] Cocoa nibs are obtained by breaking cocoa beans and removing shell particles by winnowing.[22,34,35] The shell is removed because it contains a high bacterial and pesticide load, is difficult to process, and does not contribute to the desired flavor.[34,35] After pre-drying, cocoa beans are roasted. Roasting is an essential step for flavor development and contributes significantly to the typical flavor.[24,31] Cocoa can be roasted as whole beans, nibs, or liquid cocoa mass.

Roasting temperatures between 110 and 140 °C are common.[22–24,34,35] Temperature and time vary depending on the cocoa product and the desired flavor profile.[24,35] If the temperature is too high or the roasting time is too long, cocoa develops a burnt odor and is considered over-roasted.[24] In addition to flavor, roasting is also important for product safety. It reduces the moisture content to about 2%, reduces the total bacterial count, and kills pathogenic microorganisms.[22,24,35] Cocoa liquor is obtained by grinding cocoa nibs.[22,34,35] Several grinding steps are necessary to obtain the desired particle size of 15–70 µm.[34] Coarse pre-grinding is carried out with disk mills, hammer mills, or pin mills. Fine grinding is most commonly done with five-roll roller mills.[34,35] The cocoa butter melts due to the frictional heat, causing the material to liquefy.[22,23] The cocoa liquor obtained can either be used directly for chocolate production or the production of cocoa butter and cocoa powder.[34] The cocoa mass contains 47–56% fat, which can be partially extracted by mechanical pressing. The obtained cocoa butter is partially or fully steam deodorized and optionally mixed with crude cocoa butter, depending on the intended use.[34,35] For the production of dark chocolate, cocoa butter with more flavor is usually used.[35] The remaining cocoa press cake is ground into cocoa powder by pin or hammer mills.[34,35]

Optionally, cocoa may be treated with an alkaline solution like potassium or sodium carbonate.[22] Alkalization improves the release of cocoa butter during pressing and enhances the wettability of cocoa powder. Further, alkalization results in a darker color and an improved flavor.[22,24,34,35] For the production of chocolate, cocoa mass is mixed with sugar and cocoa butter (Figure 7).[22,34] After pre-grinding in a two-roll refiner, the mixture is refined in a five-roll refiner.[22,24,34] Refining is important for a smooth texture associated with particle sizes of < 30 µm.[22] The obtained chocolate mass is mechanically treated by conching. During conching, the mass is continuously mixed, causing the temperature to rise to around 60 °C due to inner friction.[22–24,34] The chocolate mass becomes more liquid over time, and in the final stage of conching, additional cocoa butter is added to further improve the flow properties.[24,34] The solid particles get covered by fat, which provides the chocolate with the desired texture.[23] With modern equipment, conching lasts 6–24 hours and apart from texture, also improves flavor.[22–24,34] In the next step, the chocolate mass is tempered to achieve an optimal texture and appearance during storage. Tempering aims at a specific fat crystal structure.[22,34] The liquid chocolate mass is first cooled to 32 °C and then to 27–27.5 °C under stirring.[34] To melt any unstable crystals, the temperature is raised to 29–31 °C before molding.[34] The molded chocolate is finally cooled to 7–15 °C to obtain solid chocolate bars.[22]

The quality of chocolate depends highly on the cocoa beans used as raw material. Cocoa beans for industrial mass chocolate production are often blends from different origins. Blending is done before or after roasting to achieve a consistent product quality with desired flavor characteristics.[21,34,35] The blends are usually made with Forastero beans of bulk quality.[21,22]

In contrast to most industrially produced chocolates, small batch chocolates are usually made with fine flavor cocoa from a specific origin and variety.[29,36] The bean-to-bar chocolates are produced in small quantities, typically 2–60 kg chocolate per week, and meet the consumer's demands for high-quality, sustainable, and organic bean-to-bar chocolates.[22,29,36] These chocolates typically differ from mass-market chocolates by a unique flavor profile.[37,38]

In addition to the traditional chocolate making process described above, a novel processing technology has recently been introduced (Figure 8).[38–40]

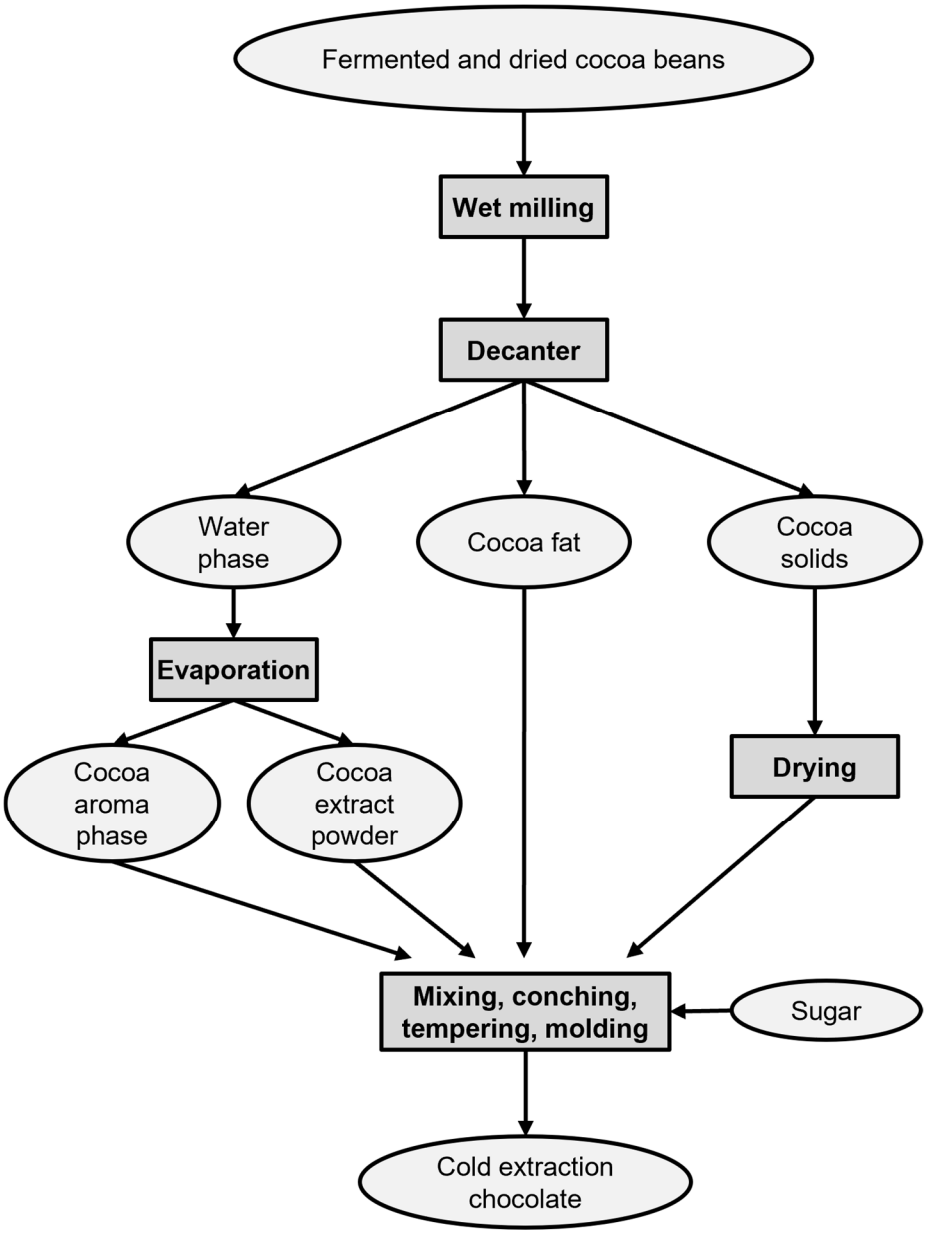

Figure 8: Simplified illustration of the novel chocolate processing approach

In the first step, fermented and dried cocoa beans are ground with water in a ratio of 1:3. The slurry is then separated into fat phase, water phase, and cocoa solids in a three-phase-decanter. The water phase contains polyphenols and odorants. It is further treated by distillation to obtain an aroma fraction and a cocoa extract powder rich in cocoa polyphenols. The four different phases can be stored and finally recombined into a chocolate mass. As in traditional chocolate making, the mass is treated by conching, tempering, and molding to manufacture the chocolate. In contrast to traditional chocolate processing, water is applied in the novel process. In addition, the beans are not roasted, and the entire process is carried out under gentle temperature conditions (≤ 60 °C).[38] Chocolates made with the novel processing technology (NPCs) differ in flavor from chocolates made with the traditional processing technology (TPCs) and were characterized by a high aroma intensity and complexity with

dominating flower and fruit notes. Furthermore, they are lower in acidity and bitterness than TPCs.[38]

4.2.4 Odor-active compounds in cocoa and chocolate

Flavor is an important quality aspect of cocoa and chocolate.[21,22] The molecular background of the odor as part of the flavor has been the subject of numerous studies.[41–45] About 600 volatiles have been found in cocoa and chocolate,[23,24] but only 20 to 30 compounds have been shown to contribute to the unique odor.[38,44–47] The chemical structures of important cocoa and chocolate odorants are shown in Figure 9.

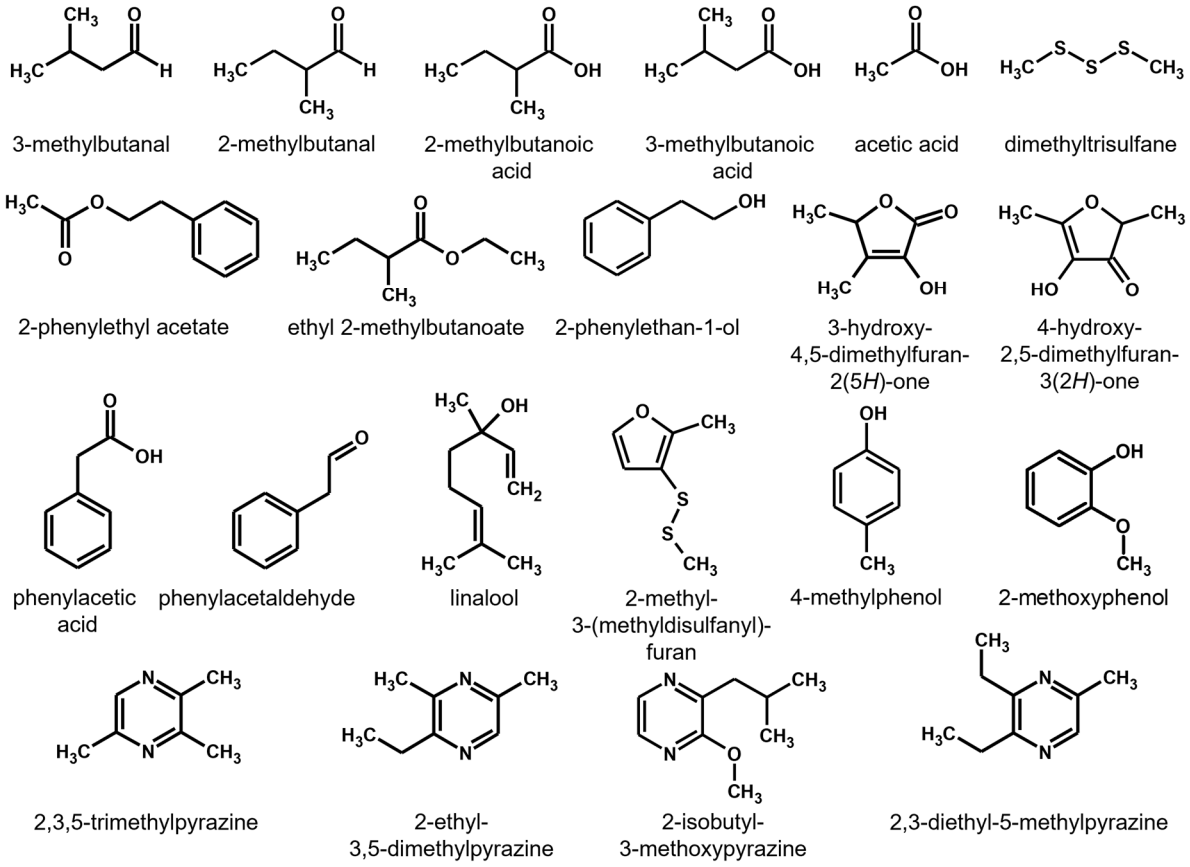

Figure 9: Structures of selected cocoa and chocolate key odorants[38,44–47]

The most important steps for flavor formation during chocolate making are fermentation, drying, roasting, and conching.[24,33] Although the typical odor predominantly develops during these processing steps, many key odorants are already present in small amounts in unfermented cocoa beans.[48,49] Unfermented cocoa has an acidic taste, but a flat aroma.[23] During fermentation and drying, the concentrations of most odor-active compounds increase. Important odorants formed during fermentation and drying are aldehydes, acids, alcohols, and esters.[24] An overview of odor-active compounds derived from amino acids during fermentation is shown in Table 1. Aldehydes such as 2- and 3-methylbutanal are formed from the parent amino acids by Strecker degradation.[24,48] These are partially converted into acids and alcohols.[24] High amounts of volatile acids such as acetic acid, butanoic acid, and 2- and 3-methylbutanoic acid are formed during fermentation.[31,48] Important odor-active alcohols are

3-methylbutan-1-ol and 2-phenylethan-1-ol.[32] In addition, alcohols are esterified with acids to odor-active esters such as 2-phenylethyl acetate, ethyl 2-methylbutanoate, and ethyl 3-methylbutanoate.[24,48] Alcohols, fatty acids, and esters are mostly formed by yeasts.[32]

Table 1: Aldehydes, acids, alcohols, and esters derived from amino acids during fermentation[24]

Amino acid	Aldehyde	Acid	Alcohol	Ester
Leucine	3-Methylbutanal	3-Methylbutanoic acid	3-Methylbutan-1-ol	3-Methylbutyl acetate Ethyl 3-methylbutanoate
Isoleucine	2-Methylbutanal	2-Methylbutanoic acid	2-Methylbutan-1-ol	2-Methylbutyl acetate Ethyl 2-methylbutanoate
Phenylalanine	Phenylacetaldehyde	Phenylacetic acid	2-Phenylethan-1-ol	2-Phenylethyl acetate
Valine	2-Methylpropanal	2-Methylpropanoic acid	2-Methylpropan-1-ol	2-Methylpropyl acetate Ethyl 2-methylpropanoate
Alanine	Acetaldehyde	Acetic acid	Ethanol	Ethyl acetate

Drying reduces the concentrations of odor-active, volatile acids but excessive heat and rapid drying lead to an insufficient loss of these unpleasant-smelling odorants.[24] After fermentation and drying, acetic acid is the odorant with the highest concentration and odor activity.[23,46–48] It further contributes to the acidity of fermented and dried cocoa beans.[23]

In addition to the formation of odorants, odor precursors are formed during fermentation. The formation of acetic acid lowers the pH to 4–5, which leads to the release of bean compounds such as sugars and proteins from their compartments.[23] Consequently, they come into contact with enzymes. The enzymatic degradation yields reducing sugars as well as oligopeptides and amino acids, which result mainly from the proteolysis of globulins.[21,23,31,33] Especially amino acids with hydrophobic side chains such as leucine, isoleucine, phenylalanine, and valine are important flavor precursors. The type and extent of the precursors formed depend strongly on the enzymatic activity. The enzymatic activity is controlled by the pH during fermentation, which mainly depends on the concentration of acetic acid.[23,24] Reducing sugars and amino acids or oligopeptides are the reactants of the Maillard reaction, which is responsible for the formation of several cocoa key odorants during roasting.[23] The concentration of reducing sugars is crucial, as they are converted to a greater extent than the amino acids, which are additionally recycled during the Maillard reaction.[24]

First, a reducing sugar such as glucose reacts with an amine such as an amino acid or a peptide to form an imine or Schiff base.[22,50,51] The Schiff base is transferred to a 1,2-enaminol by tautomerization.[22,51] In the case of glucose as the reducing sugar, the 1,2-enaminol is rearranged to an Amadori compound, which is an important intermediate of the Maillard reaction.[24,50,51] Amadori products as one of the first intermediates of the Maillard reaction are formed in considerable amounts already during fermentation and drying. They are important precursors for various odorants such as Strecker aldehydes formed during roasting.[23,24,49,52] The Amadori compound can rearrange to a 1,2-enaminol or a 2,3-enaminol. These compounds are further converted into deoxyosones. The amino compound is released again and available for further reactions.[50,53] Deoxyosones are α-dicarbonyl compounds and important educts in other reactions like the Strecker degradation.[50] In the Strecker reaction, an α-dicarbonyl

compound reacts with an amino acid to form the Strecker aldehyde and an aminoketone.[50,54] Strecker aldehydes can also be formed directly by oxidative degradation of the corresponding Amadori compounds.[49,55,56] In addition to Strecker aldehydes, the Strecker reaction also yields the corresponding Strecker acids.[48,54] The ratio of aldehydes and acids depends on reaction parameters such as pH, the presence of oxygen, and the structure of the α-dicarbonyl compound involved in the reaction.[54] Further odorants, especially heterocyclic compounds such as pyrazines and furanones, are generated in follow-up reactions of the Maillard and Strecker reactions.[22,51]

Roasting develops the typical cocoa flavor by the described processes,[24] but changes the odorant profile only quantitatively, not qualitatively.[46,47] Frauendorfer and Schieberle[45] found 24 compounds with an OAV >1 in roasted cocoa powder. The most important odorants according to the OAVs were acetic acid, 3-methylbutanal, 3-methylbutanoic acid, phenylacetaldehyde, and 2-methylbutanal.[45] 3-Methylbutanal has very early been identified as a key contributor to the flavor of roasted cocoa.[57] The Strecker aldehydes 2- and 3-methylbutanal, and phenylacetaldehyde are formed in considerable amounts during roasting by Strecker degradation of the corresponding amino acids.[23,24,46–48] In addition, the concentrations of the Strecker acids 2- and 3-methylbutanoic acid increase during roasting.[48,54] Other important compounds in roasted cocoa are pyrazines.[23,24,58] The concentrations of the pyrazines 2,3-diethyl-5-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine increase during roasting, but the impact of the pyrazines on the overall odor is low.[23,45,47,48] Furthermore, the caramel-like smelling 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one is formed in considerable amounts during roasting.[46,47] Finally, roasting leads to an increase in the concentration of most cocoa key odorants. However, the concentrations of some odorants, such as acetic acid and esters, are reduced by the high temperatures of roasting.[46–48] The loss of volatile acids is associated with reduced acidity.[22] Although its concentration decreases during roasting, acetic acid shows the highest concentration and OAV among the key odorants in roasted cocoa.[45,47]

Further steps in chocolate processing influence the odorant profile of the final chocolate. Conching partially removes undesirable volatile acids such as acetic acid and 2- and 3-methylbutanoic acid, and some phenols, which leads to an improved odor.[22–24,34] In addition, the odor is improved by a more uniform redistribution of odorants which are partly transferred to the sugar surfaces.[24] The concentrations of few odorants such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one increase during conching.[59] However, desirable odorants such as Strecker aldehydes and alcohols are partially evaporated.[23,24,59] Alkalization partially neutralizes volatile acids, which leads to an improved flavor.[23] Furthermore, alkalization leads to higher concentrations of alkylpyrazines, aldehydes, nitrogen compounds, and sulfur compounds, as the rate of Maillard reaction increases at higher pH.[24]

The first studies on chocolate odorants using AEDA were carried out by Schnermann and Schieberle[42] with milk chocolate and cocoa mass[42] and by Schieberle and Pfnuer[41] with bitter chocolate and milk chocolate. They showed that 3-methylbutanal is a key odorant in chocolate.[42] Like in roasted cocoa, further important odorants in dark chocolate are acetic acid, 3-methylbutanoic acid, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, dimethyltrisulfane, phenylacetic acid, 2-ethyl-3,5-dimethylpyrazine, and 2-methoxyphenol.[38,44] The odorant profile of milk

chocolate differs from that of dark chocolate, and ingredients like vanillin can be used to modify the odor of chocolate.[42]

Chocolates produced by the novel process (cf. section 4.2.3) have different odorant profiles than the TPCs.[38] In brief, NPCs were characterized by higher OAVs of fruity smelling esters such as ethyl 2-methylbutanoate and ethyl 3-methylbutanoate, and drastically lower concentrations of the volatile acids acetic acid, 2- and 3-methylbutanoic acid and 2-methylpropanoic acid compared to the TPCs. Furthermore, the OAVs of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, and 2-phenylethanol were higher in the TPCs, whereas the OAVs of 2-methoxyphenol and phenylacetic acid were higher in the NPCs. Interestingly, no clear differences were found in the concentrations of compounds known from the literature to be mainly formed by thermal treatment, such as Strecker aldehydes, pyrazines, and lipid oxidation products.

Some odorants that cause off-flavors have been identified in cocoa and chocolate. Drying with fire can lead to a smoky off-flavor when the smoke comes into contact with the beans and volatile phenols are absorbed.[23,24] The phenols responsible for a smoky off-flavor are namely 2-methoxyphenol, 4-methylphenol, 3-ethylphenol, 3-methylphenol, 4-ethylphenol, and 3-propylphenol.[60] A moldy-musty off-flavor was mainly associated with (–)-geosmin and 3-methyl-1*H*-indole.[61] In addition, an investigation on the molecular background of an atypical coconut-like odor revealed (*R*)-δ-2-decenolactone as the key contributor.[62]

4.2.5 Taste-active compounds in cocoa and chocolate

Besides odor, taste is an important contributor to flavor. Humans can differentiate between at least five taste modalities, namely sweet, bitter, sour, salty, and umami. Taste-active compounds are perceived by the taste buds in the taste papillae on the tongue. Each taste bud contains 50–100 taste cells, and the tastants can interact with taste receptors on the taste cells or with ion channels. The type of interaction depends on the taste modality.[11] The taste of roasted cocoa is usually described as a balanced bitterness with a typical astringent mouthfeel and a slightly sour taste. These taste properties are important for the overall sensory impression of cocoa-containing products.[43]

The key tastants in roasted cocoa nibs have been identified by Stark et al.[43] by quantitation of taste-active compounds and calculation of dose over threshold (DoT) factors. As an equivalent to the OAV of an odorant, the DoT factor is defined as the ratio between the concentration and the taste detection threshold of a tastant.[43,63] The authors verified their results with taste recombination and omission experiments.[43] Theobromine, caffeine, and diketopiperazines were evaluated as the main contributors to the bitter taste of roasted cocoa.[23,43] According to the DoT factors, theobromine has the highest impact on bitter taste, followed by caffeine and the 2,5-diketopiperazine cyclo(L-Pro-L-Val).[43] Although cyclo(L-Pro-L-Val) was the only diketopiperazine with a DoT factor >1, the omission experiments revealed the importance of the entire group of diketopiperazines for a balanced bitter sensation. The flavan-3-ol monomers catechin and epicatechin, and the oligomeric procyanidins were identified as key flavor compounds that contribute to both, bitter taste and astringency.[23,24,43,64] Epicatechin showed the highest DoT factor for bitter taste within this group, and all flavan-3-ol concentrations exceeded their threshold concentrations for astringency. Polyphenol

glycosides, *N*-phenylpropenoyl amino acids, γ-aminobutyric acid, and β-aminoisobutyric acid were found to have astringent and mouth-drying effects without any bitter taste. Among the sour tasting compounds, citric acid and acetic acid showed the highest DoT factors.[43] Thus, acetic acid contributes to both, the acidic odor and the sour taste of cocoa and chocolate.

Like the cocoa odor, the typical cocoa taste is also formed during fermentation, drying, roasting, and conching.[24,33] During fermentation and drying, procyanidin monomers and oligomers are enzymatically oxidized and polymerized.[23,31] The lowered concentrations of these taste-active compounds reduce bitterness and astringency compared to unfermented cocoa beans.[21,23,65] Acetic acid and lactic acid, which contribute to the sour taste, diffuse into the beans during fermentation.[23] Cocoa beans initially contain 1–3% theobromine and 0.1–0.2% caffeine.[24] The concentrations of these bitter tasting alkaloids are chemically not affected by fermentation, drying, and roasting.[23,24] However, part of the methylxanthines is lost from the cotyledons by diffusion.[21]

Roasting leads to a further decrease of the concentrations of procyanidin monomers and oligomers such as epicatechin, procyanidin B2, and procyanidin C1.[65–67] In contrast, roasting leads to an increase in the concentrations of bitter tasting diketopiperazines. Diketopiperazines are cyclic dipeptides generated from peptides or amino acids formed during fermentation.[23,24,66,68] Alkalization of roasted cocoa reduces astringency by oxidation and polymerization of monomeric flavonoids, mainly epicatechin.[23,24,65]

4.2.6 Influence of water on odor-active compounds in cocoa and chocolate

The addition of water has been shown to affect the concentrations of odor-active compounds in cocoa and other foods.[41,69–73] In an early study in 1999, Schieberle and Pfnuer[41] compared odor-active compounds in milk chocolate extracted by simultaneous steam distillation/extraction (SDE) with water-free extraction using a low boiling solvent followed by gentle high vacuum distillation (HVD). They observed higher FD factors of the Strecker aldehydes phenylacetaldehyde, 3-methylbutanal, and 2-methylpropanal in the SDE extract than in the HVD extract. Quantitation revealed that the concentration of phenylacetaldehyde obtained after SDE was 120-fold higher than that after HVD. They suggested the presence of specific precursors in the chocolate that released the Strecker aldehydes, e.g. by hydrolytic cleavage during SDE.[41]

Later, the impact of water on roasted cocoa and chocolate was studied more intensively.[71,72] Buhr et al.[72] observed a tenfold increase in the concentrations of the Strecker aldehydes 2- and 3-methylbutanal when roasted cocoa beans were treated with water. The concentration of phenylacetaldehyde increased 47-fold. The concentrations also increased when the solvent extract was treated with water. They concluded that the Strecker aldehydes were probably released by water from specific precursors. The authors suggested the precursors to be intermediates of the Strecker reaction, which are stable in dry cocoa beans. When water is available during sample work-up or salivation, the Strecker aldehydes are released from the precursors.[72]

Granvogl et al.[71] showed with proton transfer reaction-mass spectrometry (PTR-MS) measurements that 3-methylbutanal is generated from precursors in chocolate after the addition of water or saliva. The observed increases by saliva were 43-fold for methylpropanal,

8.5-fold for 2-methylbutanal, and 8.9-fold for 3-methylbutanal. They proposed 3-oxazolines as precursors. As suggested by Buhr et al.,[72] they hypothesized that the oxazolines are formed as part of the Strecker reaction, are stable under dry conditions, and release the Strecker aldehydes upon contact with water. Experiments with differently substituted 5-methyl-3-oxazolines confirmed them as potential precursors of 3-methybutanal, phenylacetaldehyde, 2-methylbutanal, and methylpropanal.[71] It was assumed that the generation of Strecker aldehydes from precursors in the mouth has a major impact on retronasal odor perception.[72]

4.2.7 Molecular background of fine flavor properties of cocoa and chocolate

Chocolate is a popular food that is known for its unique flavor, which is mainly determined by the cocoa beans.[24,35] Depending on their flavor quality, cocoa beans are defined as fine flavor cocoa or bulk cocoa (cf. section 4.2.1). The molecular background of the flavor of cocoa and chocolate and its development along the processing chain has been studied intensively (cf. section 4.2.4 and section 4.2.5).[24,38,44–47] However, the focus was mostly on the effect of technological processing and most studies on sensory-active compounds in cocoa products were carried out with cocoa of undefined origin, variety, and flavor characteristics.[44,45,47] Such products are usually blends from bulk-grade cocoa beans. However, single origin chocolates show different sensory properties than industrial blend chocolates.[38] Small batch chocolates are manufactured with fine flavor cocoa beans of defined origin and variety, and the flavor diversity of cocoa products of defined origin and variety has been described in several studies.[21,22,30,33,74] Fine flavor attributes described in the literature include fruity, floral, acidic, malty, cocoa, roasty, and spicy.[29,33,38]

While some off-odors have been elucidated recently,[60–62] the molecular background of fine flavor properties is not fully understood yet. First attempts have been made to identify the odor-active compounds that are responsible for the sensory properties of fine flavor cocoa and chocolate by combining sensory and instrumental methods.

Linalool was found in high concentrations in flavor-grade cocoa beans and assumed to be essential for the floral, leafy, and tea-like odor notes in cocoa.[23,75] In a study that compared Nacional and CCN51 cocoa, a higher intensity of floral and honey-like notes in the Nacional cocoa went along with higher concentrations of phenylacetaldehyde, linalool, 2-phenylethan-1-ol, 2-phenylethyl acetate and ethyl phenylacetate.[76] The more intense malty notes in the Nacional samples were in line with higher concentrations of 2- and 3-methylbutanal.[76] Liu et al.[77] analyzed the odor-active compounds of two chocolates and a cocoa liquor with GC-O and GC-MS. The malty odor perceived during sensory evaluation was associated with high concentrations of 2-methylpropanal, 3-methylbutanal, and volatile carboxylic acids. Floral odor notes were associated with the compounds phenylacetaldehyde and 2-phenylethan-1-ol. A caramel-like odor was linked to 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and maltol, whereas a coconut-like odor was linked to lactones. No compounds could be associated with a fruity odor in this study. Another study associated the sensory properties of dark chocolates with certain odorants based on GC-O analyses. However, the sensory properties were not specified in the study.[78] Rottiers et al.[79] analyzed cocoa liquors of four EET cultivars and one CCN51 sample. They performed a sensory characterization and a semi-quantitation of volatiles with headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-

GC-MS). The obtained OAVs suggested a broad range of compounds to be responsible for fruity, floral, chocolate/nutty, and buttery/creamy odors. The fruity odor described during the sensory evaluation was linked to several volatiles. They concluded that flavor perceptions are the results of complex interactions of volatiles and their balance, and that masking and synergistic effects of volatiles play an important role in flavor perception.[79] This is in line with Sukha et al.[74] who assumed that all well-fermented cocoa beans show a basic cocoa flavor which can be easily masked by other flavor attributes such as fruity, floral, and acidic.

4.2.8 Impact of the cocoa bean variety on the flavor of cocoa and chocolate

Even though fine flavor cocoa is mainly distinguished from bulk cocoa by the flavor quality, the genetic variety is another criterion (cf. 4.2.1). With exceptions, it is often generalized that fine flavor cocoa comes from Criollo, Trinitario, and Nacional cocoa trees, while bulk cocoa comes from Forastero trees.[21–23,29,30] In addition, certain countries have been classified as exclusive or partial exporters of fine flavor cocoa, and studies typically differentiate between fine flavor and bulk cocoa by origin.[30,74] However, the genotype is no guarantee of high flavor quality, and both, good and poor flavor properties can be found within one variety.[30]

Certain varieties and origins of cocoa are associated with specific flavor qualities in the literature.[30,33,80,81] Criollo and Trinitario cocoas are known to produce a fine chocolate flavor that is often described as mild nutty and full flavor.[24] Criollo beans from Venezuela are characterized by nutty, malty, and caramel notes.[30] Nacional cocoa from Ecuador is described as having floral, honey-like, and malty notes.[30,76] Chocolates made of Nacional cocoa beans have a typical flavor with mild, floral, and earthy notes.[24] Chocolates made from beans from West Africa such as Nigeria, Ghana, and Côte d'Ivoire have been described as having a strong cocoa and chocolate-like flavor with nutty notes.[22,74,81] Beans from Trinidad and Tobago and Papua New Guinea show distinct fruity and acidic flavor properties after fermentation and roasting.[30] Likewise, local Trinitario clones from Trinidad were described as having pronounced fruity and acidic flavor notes.[74]

First attempts have been made to characterize the influence of the variety on the flavor composition of cocoa products on a chemical level. The ratio of the bitter tasting compounds theobromine/caffeine has been shown to depend on the cocoa bean variety and origin.[30,31] Furthermore, the bitter tasting 2,5-diketopiperazines were found in higher concentrations in Forastero chocolates than in Criollo chocolates.[82] Quin et al.[83] analyzed the volatile composition of 16 samples of unfermented cocoa beans of the varieties Criollo, Forastero, and Trinitario. The results showed an influence of the genetic background on the volatile composition of the samples. Trinitario cocoa showed higher concentrations of most chemical compound classes such as acids, alcohols, and esters than Criollo cocoa. The concentrations of these compounds in Forastero cocoa were very low. Tuenter et al.[84] compared cocoa liquor and chocolate from Nacional cocoa with those from blended bulk cocoa beans from West Africa. They analyzed the volatile compounds and found clear differences between the samples. The Nacional samples were associated with 2-phenylethan-1-ol among other compounds. The West African blend liquor was associated with 2- and 3-methylbutanal together with roasty smelling compounds such as pyrazines. Amores et al.[30] suggested pyrazines and other volatiles as potential indicators for differentiating samples of different

flavor qualities and different origins. In another study, it was found that the linalool concentration depends on the country of origin. While high concentrations were found in fine-grade cocoa beans from Ecuador, Venezuela, and Trinidad, bulk cocoa beans contained only small amounts of linalool.[75]

Even though cocoa products of defined variety and origin have already been studied intensively, most studies only performed sensory analysis[74] or analyzed the total volatile composition without focusing specifically on flavor-active compounds.[83,85–88] Studies that evaluated the impact of the volatiles on odor by calculating OAVs mainly used semi-quantitative methods like headspace analyses, which do not cover all known cocoa key odorants.[79,84,89] Studies that analyzed odor-active compounds were mostly carried out with products made from cocoa of undefined variety.[38,42,44,45] In contrast, the investigations of Frauendorfer and Schieberle[46,47] focused on sensory-active compounds and analyzed cocoa beans of defined variety. They compared the odorant profiles of unroasted and roasted Criollo and Forastero beans. In general, the differences were not very pronounced. However, considerable differences were found for 2-heptanol, a δ-octenolactone, and linalool. With 640 µg/kg in the roasted beans, Forastero cocoa showed a higher concentration of linalool than the Criollo cocoa (130 µg/kg in the roasted sample). The concentration of a δ-octenolactone was also higher in the Forastero sample.[46,47] In conclusion, the influence of the cocoa variety on the flavor compound composition of chocolate has not been comprehensively investigated yet.

5 Objectives

The importance of the roasting step for the flavor development of cocoa has been shown in several studies. In a novel chocolate processing technology, fermented and dried cocoa beans are not roasted but treated with water. The chocolates made by the novel processing technology (NPCs) show similar concentrations of certain compounds as the chocolates made by the traditional processing technology (TPCs). This includes compounds known to be mainly formed during roasting, such as Strecker aldehydes and pyrazines. These compounds may be released during the water treatment step in the novel process. Water has already been shown to release Strecker aldehydes from precursors in roasted cocoa and chocolate, but this has not yet been shown for fermented and dried cocoa beans. The first aim of this work was to clarify if Strecker aldehydes and other cocoa key odorants are released from fermented and dried cocoa beans upon water contact by comparing the concentrations before and after water treatment. In addition, the impact of water on an NPC and a TPC made from the same batch of cocoa beans was studied.

Especially chocolates made from cocoa beans of defined varieties and origins are known for their unique flavor profiles including distinct fine flavor properties. Although the molecular background of cocoa and chocolate flavor has generally been well studied, fine flavors have not yet been fully understood. The second objective of this work was accordingly to decode selected fine flavor attributes of dark chocolates on the molecular level. The molecular bases of the fine flavor properties fruity and acidic, cocoa-like and roasty, and floral and astringent were elucidated in six chocolates by means of AEDA, quantitation of flavor-active compounds, and calculation of DoT factors.

In the literature, fine flavor properties are mainly associated with cocoa products of a single variety or origin. Furthermore, the variety is often used as an indicator to distinguish fine flavor cocoa from bulk cocoa. However, the impact of cocoa bean variety on the flavor compound profile of cocoa and chocolate has not yet been comprehensively studied. In the third part of this work, the influence of the cocoa bean variety on the flavor compound compositions of dark chocolates was studied. Ten additional single variety chocolates were analyzed and the DoT factors of key flavor compounds in a total of 16 chocolates from Forastero, Trinitario, Criollo, and Nacional cocoa beans were compared.

6 Results and discussion

6.1 Impact of water

6.1.1 Impact of water on odor-active compounds in fermented and dried cocoa beans

The release of Strecker aldehydes in roasted cocoa products by water has been demonstrated in previous studies.[71,72] However, it was not known how contact with water affects Strecker aldehydes and other important cocoa key odorants in fermented and dried cocoa beans. We investigated the impact of water in fermented and dried cocoa beans from Costa Rica (Trinitario) by comparing the concentrations of 33 odor-active compounds before and after water treatment.[90] The concentrations before water treatment were determined without a water treatment of the sample before the work-up. The concentrations after water treatment were determined by treating the ground cocoa nibs with twice the amount of water at 36 °C for 10 min before the work-up. The water was removed again by anhydrous sodium sulfate prior to the sample work-up. The water treatment parameters were selected to ensure the exhaustive release of Strecker aldehydes.[90] The sample work-up consisted of solvent extraction with dichloromethane or diethyl ether, SAFE, and concentration of the distillate to 300 µL. Isotopically substituted odorants were added as internal standards at the beginning of the extraction period. Concentrates were analyzed by GC-MS and odorant concentrations were calculated from the peak areas of standard and analyte, the amount of standard added, and the sample weight, considering individual five-point calibrations for each odorant. Odor activity values were then calculated from the concentrations in the cocoa beans and OTVs in sunflower oil or deodorized cocoa butter. Data obtained for odorants with OAVs >1 are summarized in Table 2.

The concentrations of most odorants increased after water treatment of the fermented and dried cocoa beans. The highest impact was observed for the two Strecker aldehydes 3-(methylsulfanyl)propanal (66-fold increase) and phenylacetaldehyde (50-fold increase) (Figure 10).[90] A moderate 4.4-fold increase was found for 3-methylbutanal. The increase of phenylacetaldehyde was similar to that found by Buhr et al.[72] in roasted cocoa beans (47-fold). Our data suggested that the Strecker aldehydes were formed during water treatment from precursors present in the fermented and dried cocoa beans. Previous studies already indicated the release of Strecker aldehydes by water from precursors in roasted cocoa products.[71,72] The authors assumed that these precursors are formed as part of the Strecker reaction during thermal processing.[71,72] It is not yet clear whether the same precursor structures could be present in fermented and dried cocoa beans before roasting. Alternatively, Amadori products could be involved in the nonthermal formation of Strecker aldehydes during water treatment. Amadori compounds are present in high amounts after fermentation and drying[49] and have been shown to play an important role in the thermal formation of Strecker aldehydes.[49,55] In addition, oxazolines have been hypothesized to release Strecker aldehydes during water treatment. They may be formed directly from Amadori products by oxidative decarboxylation.[71]

Table 2: Concentrations of odorants with OAVs >1 in the fermented and dried cocoa beans before and after water treatment (WT)

odorant	concentration (µg/kg)[a]		factor[b]	odor threshold value	odor activity value	
	before WT	after WT			before WT	after WT
Strecker aldehydes						
3-(methylsulfanyl)propanal[c]	5.97	394	66	0.52[e]	11	760
phenylacetaldehyde[c]	90.6	4510	50	34[e]	2.7	130
3-methylbutanal[c]	1650	7300	4.4	15[e]	110	490
2-methylbutanal[c]	1770	2250	1.3	34[e]	52	66
Strecker acids						
phenylacetic acid[c]	3920	47200	12	26[e]	150	1800
acetic acid[c]	545000	4130000	7.6	350[f]	1600	12000
3-methylbutanoic acid[c]	8090	34100	4.2	11[e]	740	3100
2-methylbutanoic acid[c]	4520	16500	3.7	110[e]	40	140
miscellaneous odorants						
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one[c]	12.5	61.8	4.9	0.20[g]	63	310
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one[c]	357	1560	4.4	27[e]	13	58
2,3,5-trimethylpyrazine[c]	420	1590	3.8	180[e]	2.3	8.8
2-phenylethan-1-ol[c]	1430	4840	3.4	490[e]	2.9	9.9
2-methoxyphenol[c]	166	406	2.4	1.8[h]	92	230
2-ethyl-3,5-dimethylpyrazine[c]	117	267	2.3	1.7[e]	69	160
4-methylphenol[c]	14.5	31.1	2.1	3.3[h]	4.4	9.4
ethyl 3-methylbutanoate[c]	40.3	77.6	1.9	0.98[e]	41	79
3-ethyl-2,5-dimethylpyrazine[c]	63.2	112	1.8	57[g]	1.1	2.0
3-ethylphenol[c]	12.5	18.0	1.4	2.2[h]	5.7	8.2
dimethyltrisulfane[c]	45.2	63.6	1.4	0.030[e]	1500	2100
linalool[c]	1010	1420	1.4	3.4[e]	300	420
2-isobutyl-3-methoxypyrazine[c]	0.356	0.445	1.3	0.041[e]	8.7	11
ethyl phenylacetate[c]	384	471	1.2	300[e]	1.3	1.6
2-methyl-3-(methyldisulfanyl)furan[c]	2.64	3.18	1.2	0.37[e]	7.1	8.6
ethyl 2-methylbutanoate[c]	35.4	39.6	1.1	0.37[e]	96	110
3-methylbutyl acetate[d]	1310	1200	0.92	76[e]	17	16

[a]Concentration values are means of triplicates; relative standard deviations were <15%. [b]Factor by which the concentration increased after water treatment. [c]Significant difference or [d]no significant difference in the concentrations of this compound before and after water treatment (α=0.05). [e]OTV according to reference 90 (in sunflower oil). [f]OTV according to reference 91 (in sunflower oil). [g]OTV according to reference 92 (in sunflower oil). [h]OTV according to reference 60 (in deodorized cocoa butter).

In traditional chocolate processing, the roasting step is thought to be mainly responsible for the formation of Strecker aldehydes.[24,46,47] However, chocolates made by a novel approach showed similar concentrations of Strecker aldehydes as TPCs, even though the cocoa beans are not roasted in the novel process.[38] Instead, the fermented and dried cocoa beans are ground after water addition at the beginning of the process. The similar concentrations might be explained by the release of Strecker aldehydes from the fermented and dried cocoa beans during water contact in the novel process.[90]

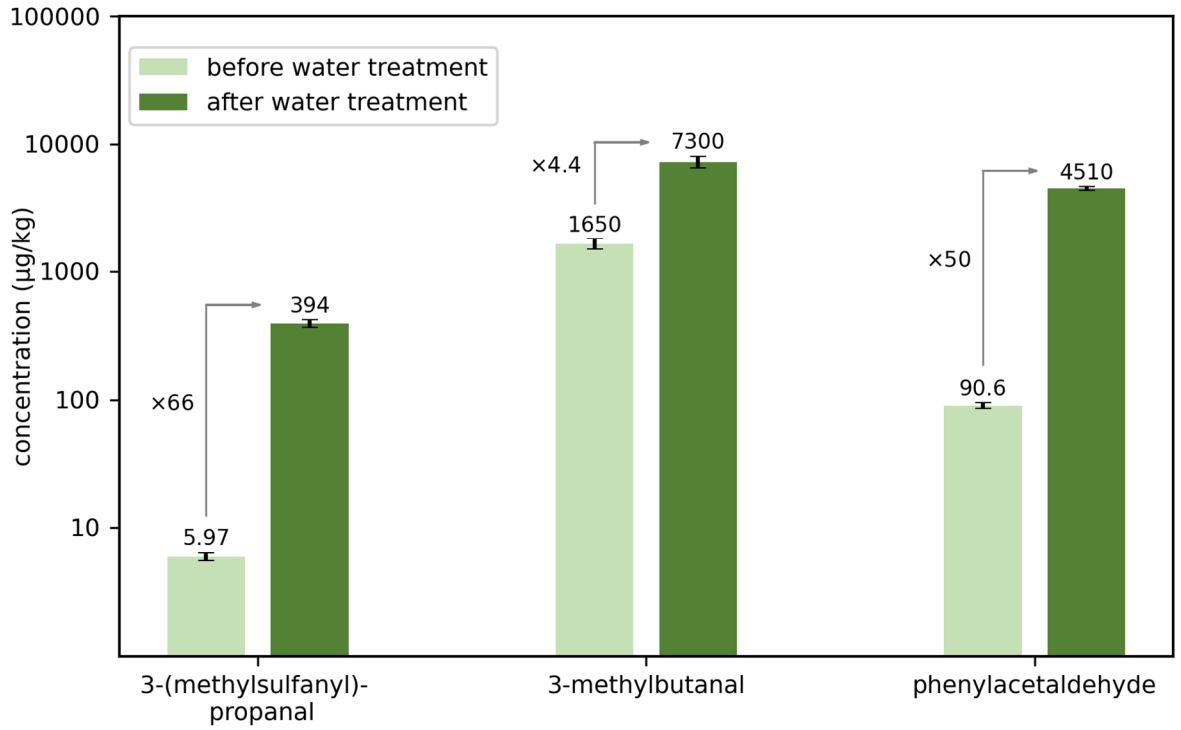

Figure 10: Concentrations of the Strecker aldehydes 3-(methylsulfanyl)propanal, 3-methylbutanal, and phenylacetaldehyde in the cocoa nibs before and after water treatment

Water treatment resulted in a remarkable increase in the concentrations of odor-active Strecker acids in the fermented and dried cocoa beans (Figure 11).[90] We observed increase factors from 3.7 for 2-methylbutanoic acid to 12 for phenylacetic acid, consistent with a specific formation via the Strecker reaction.[54] Similar to the release of Strecker aldehydes from precursors, Strecker acids may be released from yet unknown precursors in fermented and dried cocoa beans during water treatment. Another possibility is an improved extraction yield of the acids after water treatment. We assumed that the Strecker acids are present in the cocoa beans in the form of salts and are not fully extracted with a nonpolar solvent such as dichloromethane. Protonation of the carboxylates by the addition of water could lead to a higher extraction yield, which would partly explain the increase of Strecker acid concentrations after water treatment.

Finally, water treatment also led to an increase of the concentrations of most other odorants, independent of their chemical nature, although the increase factors were rather lower than those of the Strecker aldehydes and Strecker acids (Table 2). This suggested a more general mechanism than the release from specific precursors. We assumed that the odorants are noncovalently bound to cocoa matrix components. The addition of water might break the water-labile bonds, leading to higher yields in the subsequent solvent extraction.[90] Different types of interactions could occur in cocoa beans between odorants and cocoa matrix compounds such as polysaccharides, fat, proteins, and cocoa polyphenols. Some interactions have been described previously.[93] Interactions between odorants and polyphenols were observed in a cocoa bean tannin extract with a higher binding affinity of ethyl benzoate than

benzaldehyde and 2-phenylethan-1-ol.[94] These interactions were assumed to be strongly influenced by the structure of the odorant.

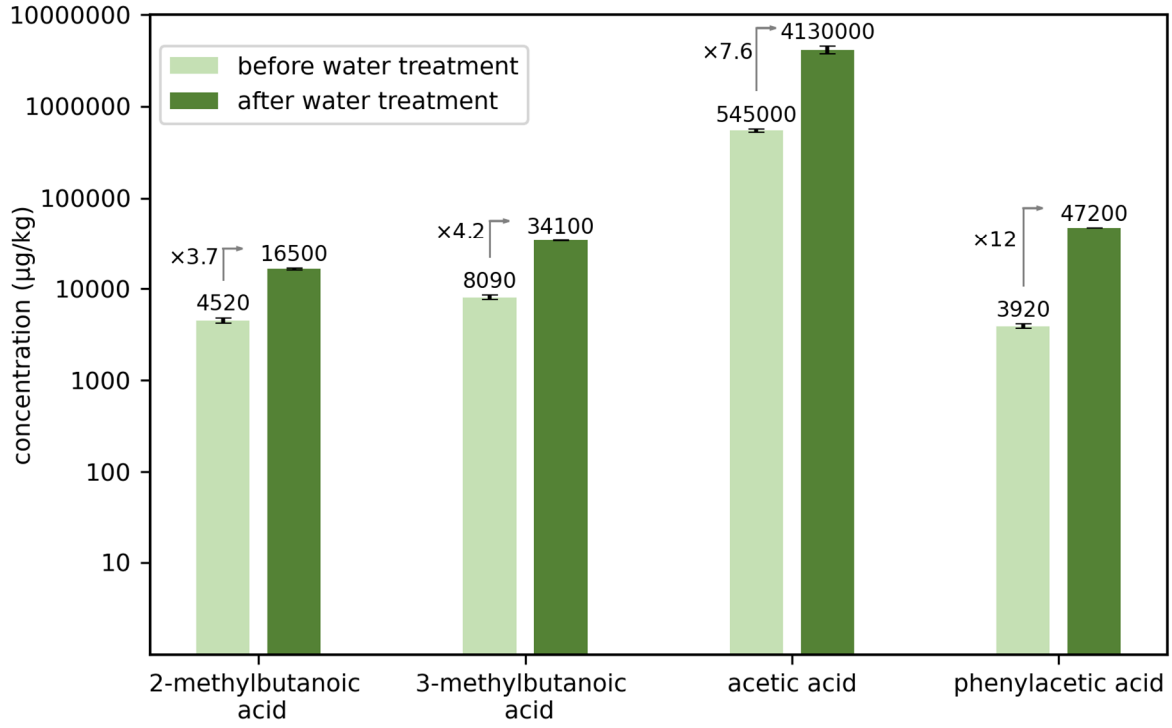

Figure 11: Concentrations of Strecker acids in the cocoa nibs before and after water treatment

The impact of water on the concentrations of odor-active compounds has been observed in other food before. The concentrations of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and 2-phenylethan-1-ol increased when hot water was added to black tea leaves.[69] The concentrations of odorants such as vanillin increased after oat extrudates were soaked in water.[73] The authors suggested that interactions of the odorants with matrix components, in particular starch inclusion complexes, were responsible for the observed increase. More recently, Rögner et al.[70] studied the impact of water on malt powder odorants. They found high increases for phenylacetaldehyde (33-fold), phenylacetic acid (14-fold), and vanillin (9-fold). They also suggested a general release mechanism in addition to the specific release of Strecker aldehydes from precursors.[70,90] However, the exact types of water-labile interactions in cocoa which are cleaved upon contact with water and release additional amounts of odorants remain to be determined.

6.1.2 Impact of water on odor-active compounds in chocolates made with two different processing technologies

Previous studies have shown that major amounts of Strecker aldehydes are released from traditionally processed chocolates, not before contact with water or saliva.[71,72] This in-mouth generation of Strecker aldehydes was assumed to play an essential role in the retronasal odor perception of chocolates.[71,72] In the novel process, the fermented and dried cocoa beans come into contact with water right at the very beginning of processing. Our data suggested that Strecker aldehydes and other odorants are released from fermented and dried cocoa beans already at this point of processing. Therefore, we expected that chocolates produced by the

novel process release significantly lower amounts of odorants upon contact with saliva than traditionally processed chocolates.[90]

We challenged this hypothesis by comparing the concentrations of selected odor-active compounds before and after water treatment in an NPC and a TPC. The selection included Strecker aldehydes, Strecker acids, and other odorants with OAVs >1 that showed high increase factors obtained from the fermented and dried cocoa beans. Both chocolates were produced from the batch of cocoa beans analyzed in section 6.1.1. However, the NPC was made on a commercial scale, while the TPC was manufactured on a laboratory scale. Our results finally showed that in most cases higher amounts of odorants were released from the NPC than from the TPC (Table 3), which actually was the opposite of what we expected.[90]

Table 3: Concentrations of selected odorants in the two differently processed chocolates before and after water treatment (WT)

	concentration in the NPC (µg/kg)[a]			concentration in the TPC (µg/kg)[a]		
odorant	before WT	after WT	factor[b]	before WT	after WT	factor[b]
Strecker aldehydes						
2-methylbutanal	39.0	1510	39	155	1130	7.3
3-methylbutanal	136	4840	36	523	4340	8.3
3-(methylsulfanyl)propanal	4.37	140	32	5.76	307	53
phenylacetaldehyde	226	5390	24	388	4150	11
Strecker acids						
acetic acid	98900	1530000	15	244000	2450000	10
3-methylbutanoic acid	831	5080	6.1	8380	16100	1.9
2-methylbutanoic acid	421	1860	4.4	4270	7020	1.6
phenylacetic acid	3360	14800	4.4	10000	26000	2.6
miscellaneous odorants						
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	2.92	24.4	8.4	15.2	49.3	3.2
4-methylphenol	12.3	34.2	2.8	18.2	31.7	1.7
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	193	520	2.7	1210	1680	1.4
2-ethyl-3,5-dimethylpyrazine	41.4	96.5	2.3	139	203	1.5
2-phenylethan-1-ol	815	1620	2.0	2010	2590	1.3
2-methoxyphenol	88.2	139	1.6	172	238	1.4
2,3,5-trimethylpyrazine	250	332	1.3	1310	885	0.67

[a]Concentration values are means of triplicates; relative standard deviations were <15%. [b]Factor by which the concentration increased after water treatment; concentrations before and after water treatment were significantly different (α=0.05) for all odorants in both NPC and TPC

The highest increase factors in the NPC were found for the Strecker aldehydes 2-methylbutanal (39-fold), 3-methylbutanal (36-fold), 3-(methylsulfanyl)propanal (32-fold), and phenylacetaldehyde (24-fold) (Figure 12). Our results indicated the presence of high amounts of Strecker aldehyde precursors in the NPC. The concentrations of all other odorants also increased after water treatment (Table 3). The increase factors were mostly higher for the NPC than for the TPC, although the release of higher amounts of odorants was expected for the TPC. We suggested that the odorants released by water contact at the beginning of the novel process may have been re-fixed later by the formation of hydro-labile complexes. These hydro-

labile interactions were probably cleaved again during water treatment of the NPC.[90] The concentrations of almost all odorants in the TPC increased after water treatment. With the exception of 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, and 4-methylphenol, higher concentrations were found in the TPC than in the NPC after water treatment. Compounds such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and 2,3,5-trimethylpyrazine are thought to be formed mainly during the roasting process[46,47] and were expected to be present in higher concentrations in the TPC. The concentrations of the Strecker acids were drastically lower in the NPC. In the novel process, the mixture of cocoa beans and water is separated in a three-phase decanter into fat phase, water phase, and cocoa solids.[38] The water phase contains high amounts of volatile, carboxylic acids such as acetic acid. The volatile acids are then efficiently removed by further treatments of the water phase such as distillation and spray drying.[38] The low concentrations of Strecker acids in the NPC can therefore be explained by their efficient removal in the novel process.

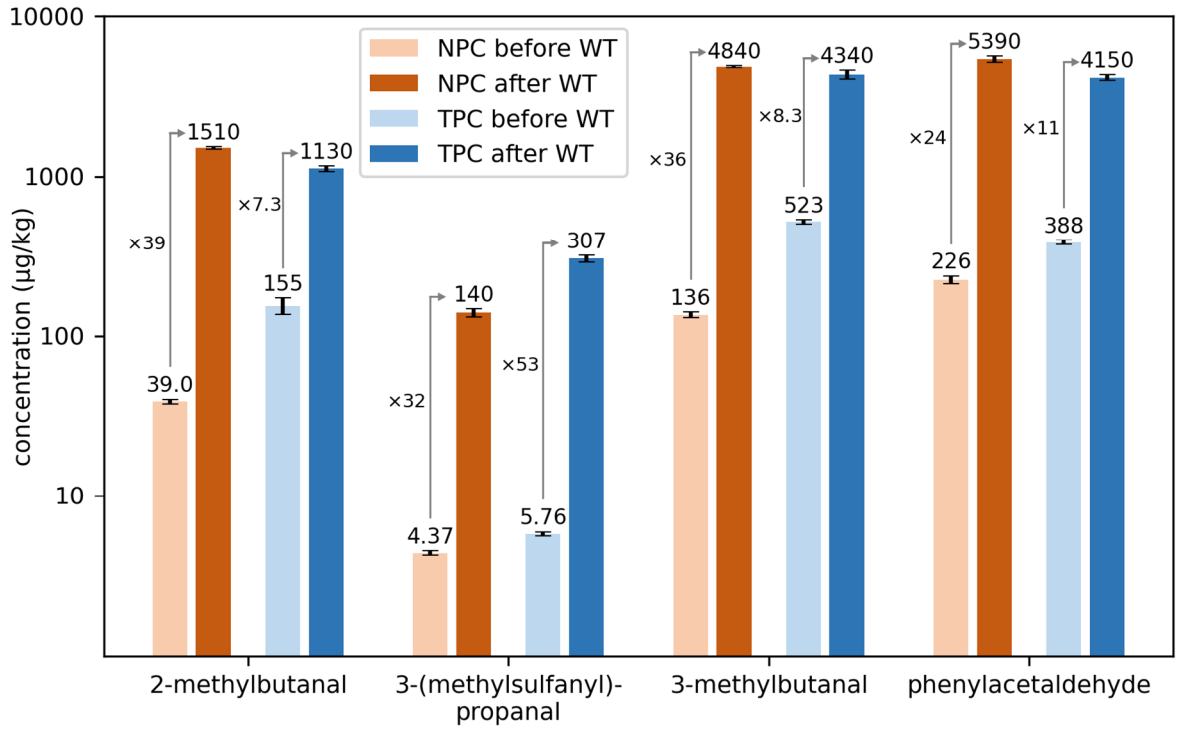

Figure 12: Concentrations of Strecker aldehydes in the chocolate made by the novel processing technology (NPC) and the traditionally processed chocolate (TPC) before and after water treatment (WT)

6.1.3 Conclusions

The impact of water on odorants has been described in previous studies for roasted cocoa and other foods.[69–73] We investigated for the first time the impact of water on odor-active compounds in fermented and dried cocoa beans.[90] Our results showed a significant impact of water on the concentrations of Strecker aldehydes, Strecker acids, and most other odor-active compounds. Furthermore, water treatment affected the odorant concentrations in chocolates made with two different processing technologies.[90] We assumed that the observed increase in odorant concentrations strongly impacts retronasal odor perception. In previous studies,

odorants were extracted from cocoa and chocolate mainly with a low boiling organic solvent, without pretreating the sample with water.[38,42,44–46] However, the release of additional amounts of Strecker aldehydes and other odorants by water or saliva is not considered if the sample is not treated with water before the work-up. The application of water treatment prior to sample work-up depends on whether the orthonasal or retronasal odor profile is to be addressed. The orthonasal odor perception is only influenced by the odorant concentrations that migrate from the matrix into the gas phase without water. The retronasal odor perception is highly impacted by the release of additional amounts of odorants by water or saliva.[7] In summary, no water treatment should be performed prior to sample work-up for the characterization of the orthonasal odor profile. However, a water treatment before sample work-up is essential to characterize the retronasal odor profile.[90]

6.2 Decoding the fine flavor properties of dark chocolates

Recent developments in the chocolate market have led to a wide variety of chocolate flavors. The diversity of fine flavor chocolates is mainly the result of a large variety of bean-to-bar products including small batch chocolates. Such chocolates are produced with traditional methods by small batch manufacturers. To obtain chocolates with unique flavor profiles, they typically use fine flavor cocoa beans of a specific variety and origin. Fine flavors have been well described at the sensory testing level,[21,22,29,30,33,74] but their molecular background has not been extensively studied.

Sensory reference samples for specific fine flavor attributes were selected by Cocoa of Excellence (CoEx) for the global use in sensory trainings and to standardize the assessment of cocoa and chocolate products. CoEx is a global platform that rewards cocoa producers from around the world for their cocoa quality and flavor diversity.[95] Every two years, CoEx awards 50 chocolates after a professional sensory evaluation of both, liquors and chocolates. A great variety of cocoa samples is evaluated and CoEx defines liquors with fine flavor characteristics as sensory reference samples for specific flavor attributes. With their distinct sensory properties, these samples have the potential to provide valuable information on the molecular background of specific fine flavor attributes.

CoEx provided chocolates produced from six reference liquors. The chocolates exhibited the same distinct flavor characteristics for which the liquors were referenced. The sensory reference attributes are listed in Table 4. With the aim of decoding the fine flavor properties of these chocolates, we performed AEDA, quantitated selected odorants and tastants, and calculated the DoT factors.

Table 4: Sensory reference attributes of the chocolates

sample code	reference attributes
Ref1	cocoa, roasty
Ref2	fruity (fresh fruit, browned fruit), acidic
Ref3	fruity (fresh fruit, browned fruit), acidic
Ref4	fruity (fresh fruit), acidic
Ref5	floral, astringent, bitter
Ref6	cocoa, roasty

6.2.1 Identification of odor-active compounds

As a first step, we performed AEDA with the three reference chocolates Ref1, Ref4, and Ref5.[96] They were chosen as representatives of the different flavor description groups (cf. Table 4). Ref1 represented samples with cocoa and roasty flavor notes, Ref4 those with pronounced fruity and acidic flavor notes, and Ref5 was described as distinctly floral, astringent, and bitter. The samples were stirred with a mixture of diethyl ether and water (volume ratio 3:1). The addition of water was selected to release additional amounts of odorants from their hydrolabile precursors, which is essential for the analysis of the retronasal odorant profile.[90] The diethyl ether phase was subjected to SAFE and the dried distillate was concentrated to a volume of 300 µL. The concentrate was diluted stepwise 1:4 up to a dilution factor of 4096 and the diluted samples were analyzed by GC-O. The identification of odor-active compounds was based on

the comparison of the RI on DB-FFAP and DB-5 columns, the odor quality perceived at the sniffing port, and the mass spectrum with data of authentic reference compounds. Among the odor-active compounds detected in at least two samples and in at least one of the samples with a minimum FD factor of 16, a total of 46 were identified (Table 5).[96]

Ref1 (cocoa, roasty) showed high FD factors of 1024 and 4096 for acetic acid, 2,3-diethyl-5-methylpyrazine, 2- and 3-methylbutanoic acid, dimethyltetrasulfane, 2-methoxyphenol, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 2-phenylethan-1-ol, ethyl cinnamate, and phenylacetic acid. The FD factors of 2,3-diethyl-5-methylpyrazine, 4-ethyl-2-methoxyphenol, 2-methoxy-4-propylphenol, and 2-methoxy-4-vinylphenol were higher in Ref1 than in the other two chocolates. 2- and 3-Methylbutanal were perceived in Ref1 with an FD factor of 64. In particular, 3-methylbutanal is known to be an important key odorant in cocoa and chocolate.[38,44,46,47] In addition to most pyrazines and phenols, higher FD factors in Ref1 than in Ref4 and Ref5 were obtained for dimethyltetrasulfane and dimethyltrisulfane, both with a sulfury note, and for the seasoning-like smelling 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one.

Ref4 (fruity, acidic) showed a very high FD factor of 1024 for the vinegar-like smelling acetic acid, suggesting that it accounted for the pronounced acidic flavor of the sample. An FD factor of 1024 was also observed for 2-methyl-3-(methyldisulfanyl)furan, 2-phenylethyl acetate, ethyl 3-phenylpropanoate, 2-phenylethan-1-ol, ethyl cinnamate, and phenylacetic acid. Furthermore, Ref4 showed higher FD factors for most fruity smelling odorants than Ref1 and Ref5, which was consistent with the intense fruity odor note of Ref4. Ref5 (floral, astringent) showed the highest FD factor of 1024 for 2- and 3-methylbutanoic acid and ethyl cinnamate. Interestingly, most odorants showed lower FD factors in Ref5 than in Ref4 and Ref1. Considering that, the FD factor of 256 for the floral smelling odorants 2-phenylethan-1-ol and 2-phenylethyl acetate indicated their importance for the intense floral odor of this sample. In summary, almost all of the detected odorants have previously been identified as cocoa and chocolate odorants.[38,44–47,60,61] Our data thus suggested that the distinct flavor properties of the reference chocolates were caused by quantitative differences of well-known cocoa and chocolate key odorants.[96]

Table 5: Odor-active compounds in the chocolate samples Ref1 Ref4, and Ref5; the listed compounds were perceived in at least two samples and in at least one sample with a minimum FD factor of 16[96]

			retention index on		flavor dilution factor[b]		
no.	compound[a]	odor quality	DB-FFAP	DB-5	Ref1	Ref4	Ref5
1	2- and 3-methylbutanal	malty	875	<700	64	16	64
2	ethyl 2-methylpropanoate[c]	fruity, apple-like	939	746	1	16	64
3	butane-2,3-dione	buttery, caramel	958	<700	64	64	256
4	methyl butanoate[c]	fruity, glue-like	974	<700	64	16	4
5	ethyl butanoate[c]	fruity	1022	803	4	16	4
6	ethyl 2-methylbutanoate[c]	fruity	1035	846	64	16	64
7	ethyl 3-methylbutanoate[c]	fruity	1056	846	16	16	1
8	3-methylbutyl acetate	banana-like, fruity	1113	878	-	16	1
9	unknown	fruity	1259	-	64	64	4
10	oct-1-en-3-one[c]	mushroom-like	1295	976	-	16	1
11	dimethyltrisulfane	cabbage-like	1357	963	64	16	4
12	2,3,5-trimethylpyrazine	earthy, roasty	1391	1000	64	256	64
13	2-methoxy-3-isopropylpyrazine[c]	earthy, green pea-like	1418	1093	64	256	64
14	acetic acid	vinegar-like, pungent	1439	<700	1024	1024	256
15	2-ethyl-3,5-dimethylpyrazine	earthy, roasty	1446	1084	256	256	64
16	2,3-diethyl-5-methylpyrazine	earthy, roasty	1477	1154	4096	256	64
17	2-methoxy-3-sec-butylpyrazine[c]	earthy, green pea-like	1486	1172	16	16	4
18	2-methoxy-3-isobutylpyrazine[c]	green bell pepper-like	1508	1180	64	256	256
19	unknown	fruity, sweaty, pungent	1513	1063	64	64	4
20	linalool	citrus-like, bergamot-like	1536	1099	16	4	4
21	2-methylpropanoic acid	cheesy, sweaty	1555	794	16	16	4
22	(2*E*,6*Z*)-nona-2,6-dienal[c]	cucumber-like, pungent	1567	1159	16	-	4
23	butanoic acid	sweaty, vomit-like, rancid	1617	820	16	16	64
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	64	1024	256

Table 5 (continued): Odor-active compounds in the chocolate samples Ref1 Ref4, and Ref5; the listed compounds were perceived in at least two samples and in at least one sample with a minimum FD factor of 16[96]

26	2- and 3-methylbutanoic acid	sweaty, cheesy	1655	859	4096	256	1024
27	(2*E*,4*E*)-nona-2,4-dienal[c]	cardboard-like, fatty, rancid	1687	1212	16	16	4
28	3-methylnonane-2,4-dione[c]	flowery, fruity, rose-like	1699	-	64	256	256
29	dimethyltetrasulfane[d]	seasoning-like, cabbage-like	1716	1212	1024	256	256
30	unknown	meaty, seasoning-like	1738	-	4	16	1
31	ethyl phenylacetate	flowery, honey-like	1773	1241	4	4	16
32	2-phenylethyl acetate	flowery, dried fruits-like	1799	1257	64	1024	256
33	2-methoxyphenol	gammon-like, smoky	1847	1087	1024	256	256
34	ethyl 3-phenylpropanoate	fruity, cinnamon-like	1868	1347	256	1024	256
35	2-phenylethan-1-ol	flowery, honey-like	1897	1111	1024	1024	256
36	trans-4,5-epoxy-(*E*)-2-decenal[d]	cardboard-like, metallic	1993	1382	16	64	256
37	γ-nonalactone	coconut-like, peach-like	2007	1362	16	16	16
38	4-ethyl-2-methoxyphenol	smoky, clove-like, spicy	2010	1274	64	-	1
39	4-methylphenol	horse stable-like	2073	1079	16	16	1
40	2-methoxy-4-propylphenol	smoky, clove-like, spicy	2094	1374	64	1	1
41	ethyl cinnamate	fruity, cinnamon-like	2114	1464	1024	1024	1024
42	γ-decalactone[c]	peach-like	2122	1469	16	-	4
43	4-ethylphenol[c]	leather-like, smoky	2155	-	16	16	16
44	3-ethylphenol[c]	horse stable-like, leather-like	2169	-	1	16	4
45	4-ethenyl-2-methoxyphenol	smoky	2184	1326	16	1	1
46	3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	seasoning-like	2186	1108	4096	256	256
47	2,6-dimethoxyphenol[c]	gammon-like, smoky	2256	-	-	1	16
48	phenylacetic acid	bees wax-like	2543	1257	1024	1024	256
49	vanillin	vanilla-like	2554	1402	4	64	16

[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; tentative identification was based on comparing the RI and the odor quality with literature data.

6.2.2 Quantitation of flavor-active compounds

As a next step towards the decoding of the fine flavor properties of the dark chocolates, we quantitated 27 odorants in all six samples. The odorants were selected based on the AEDA results and previous data in the literature. In addition, we quantitated 8 nonvolatile, taste-active compounds known from the literature.[43] Sample work-up for odorant quantitation was performed as described in section 6.2.1, but using isotopically substituted odorants as internal standards. The standards were added at the beginning of the extraction process to compensate for odorant losses during the work-up. Odorants were quantitated by GC-MS as described in section 6.1.1. The tastants were quantitated in defatted chocolate. The defatting was done by multiple *n*-hexane extraction, assuming that only insignificant amounts of the tastants were removed with the *n*-hexane extracts. Citric acid and lactic acid were extracted from the defatted chocolate with water and their concentrations were determined enzymatically. Caffeine, theobromine, (–)-epicatechin, procyanidin B2, and procyanidin C1 were extracted with acetone and water and analyzed with high performance liquid chromatography-diode array detection (HPLC-DAD).[97] Cyclo(L-pro-L-val) was extracted with ethyl acetate and analyzed by HPLC-MS/MS.[82] The obtained concentrations are listed in Table 6.[96]

Ref2, Ref3, and Ref4 showed the highest concentrations of acetic acid among all samples and predominantly higher concentrations of 2,3,5-trimethylpyrazine, 2-methoxyphenol, and esters than the other chocolates. This was in line with their distinct fruity and acidic flavor character (cf. Table 4). The concentration differences were most pronounced for 2-phenylethyl acetate and ethyl cinnamate. Among the taste-active compounds, lactic acid showed particularly high concentrations in Ref2, Ref3, and Ref4. Ref2 and Ref3, in contrast to Ref4, were additionally referenced with a browned fruit odor (cf. Table 4). The two chocolates showed higher concentrations of ethyl 3-methylbutanoate and 2-methoxyphenol than Ref4.

Ref1 and Ref6 were described as having distinct cocoa-like and roasty flavor notes (cf. Table 4). The two samples showed the highest concentrations of the Strecker aldehydes 2- and 3-methylbutanal and phenylacetaldehyde. In addition, the concentrations of 2,3-diethyl-5-methylpyrazine, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyltrisulfane were highest in Ref1 and Ref6 among all samples. Interestingly, Ref1 showed higher concentrations of most odorants than Ref6 except for acetic acid, ethyl 2-methylbutanoate, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one. Additionally, higher concentrations of the bitter tasting compounds theobromine, caffeine, and cyclo(L-pro-L-val) were obtained in Ref1 than in Ref6. The concentration of cyclo(L-pro-L-val) in Ref1 was clearly the highest among all samples and amounted to 13.7 mg/100 g. Ref5 was referenced as floral, astringent, and bitter (cf. Table 4) and showed very high concentrations of (–)-epicatechin, procyanidin B2, and procyanidin C1. Furthermore, together with Ref3 (fruity, acidic), Ref5 showed the highest concentration of 2-phenylethan-1-ol.

Table 6: Concentrations of flavor-active compounds in the six reference chocolates grouped according to their reference attributes (cf. Table 4)[96]

	concentration (µg/kg for odorants, mg/100 g for tastants)[a]					
	reference attributes					
	cocoa-like, roasty		fruity, acidic			floral, astringent, bitter
	Ref1	Ref6	Ref2	Ref3	Ref4	Ref5
odorants						
2-methylbutanal	2770	2550	897	1450	1450	1340
3-methylbutanal	11900	9300	3660	6610	4730	3480
phenylacetaldehyde	4760	3030	1600	2840	2390	1830
2-methylbutanoic acid	11000	6320	6970	7090	4730	5920
phenylacetic acid	40600	25900	16500	32700	18200	20600
3-methylbutanoic acid	23700	14400	11600	20500	11500	11200
acetic acid	1460000	1660000	2370000	2330000	3310000	1750000
ethyl 2-methylbutanoate	1.43	1.73	3.03	2.00	2.61	1.84
ethyl 3-methylbutanoate	2.27	2.06	3.41	3.75[b]	2.08	1.46
3-methylbutyl acetate	46.2	35.6	45.6	229	277	92.6
ethyl phenylacetate	178	91.8	194	790	289	270
2-phenylethyl acetate	597	496	1610	3220	2010	768
ethyl cinnamate	95.3	68.2	138	161	402	116
linalool	446	196	21.7	256	36.8	124
2-phenylethan-1-ol	5530	4970	4430	8650	5950	8650
2,3-diethyl-5-methylpyrazine	8.22	6.55	0.890	2.25	2.24	0.870
2,3,5-trimethylpyrazine	472	197	496	509	699	119
2-ethyl-3,5-dimethylpyrazine	151	61.4	111	204	109	29.6
2-methoxyphenol	88.8	19.6	171	135	88.9	35.5
4-methylphenol	21.0	12.6	9.54	25.0	40.4	10.5
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	45.3	44.2	36.5	48.5	45.4	26.0
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	4470	6310	3790	1100	1360	1280
dimethyltrisulfane	52.8	33.9	6.44	13.4	13.3	4.20
2-methyl-3-(methyldisulfanyl)furan	2.50	1.85	0.445	0.927	2.70	0.328
γ-decalactone	42.9	31.5	14.2	26.2	23.2	37.4
γ-nonalactone	595	107	78.0	308	112	369
vanillin	205	126	177	133	63.0	153
tastants						
citric acid[c]	442	605	469	317	487	337
lactic acid[c]	140	82.8	242	267	641	117
theobromine[c]	1030	922	788	1210	719	1130
caffeine[c]	121	109	216	198	155	168
(−)-epicatechin[c]	101	117	132	105	153	412
procyanidin B2[c]	66.5	92.8	97.8	63.3	87.8	242
procyanidin C1[c]	41.2	53.0	57.2	44.4	55.3	181
cyclo(L-pro-L-val)[c,d]	13.7	5.21	4.51	6.70	5.59	4.73

[a]Concentration values are means of triplicates; relative standard deviations were <15%. [b]Mean of duplicate. [c]Concentrations in the whole chocolates were calculated from the concentrations in the defatted chocolates and a fat content of 40%. [d]Values taken from a previous publication.[82]

6.2.3 Calculation of DoT factors

Quantitation of the flavor-active compounds revealed clear differences at the molecular level between the samples. However, the impact of the compounds on the sensory perception cannot be concluded from the concentrations alone. The different odor potencies of the individual odorants need to be considered additionally. Therefore, the ratio of the concentration to the sensory perception threshold was calculated for each odorant and tastant as a final step in decoding the fine flavor properties at the molecular level.[96] This ratio is usually referred to as the odor activity value (OAV) for odorants[20] and as the dose over threshold (DoT) factor for tastants.[63] Since both values are obtained by calculating the ratio of the concentration to the threshold, the term DoT factor is used for both, odorants and tastants in the following. DoT factors of all compounds with a DoT factor >1 in at least one of the samples were subjected to principal component analysis (PCA) (Figure 13).[96] The compounds 2-phenylethyl acetate, ethyl cinnamate, γ-decalactone, γ-nonalactone, and cyclo(L-pro-L-val) showed DoT factors <1 in all samples and were assumed not to play a relevant role in explaining the different flavor profiles. Three clusters were observed in the PCA plot, marked by red circles in Figure 13. The clusters were formed by chocolates with similar sensory properties. This suggested that the flavor-active compounds responsible for the different flavor profiles of the reference chocolates were included in the PCA.

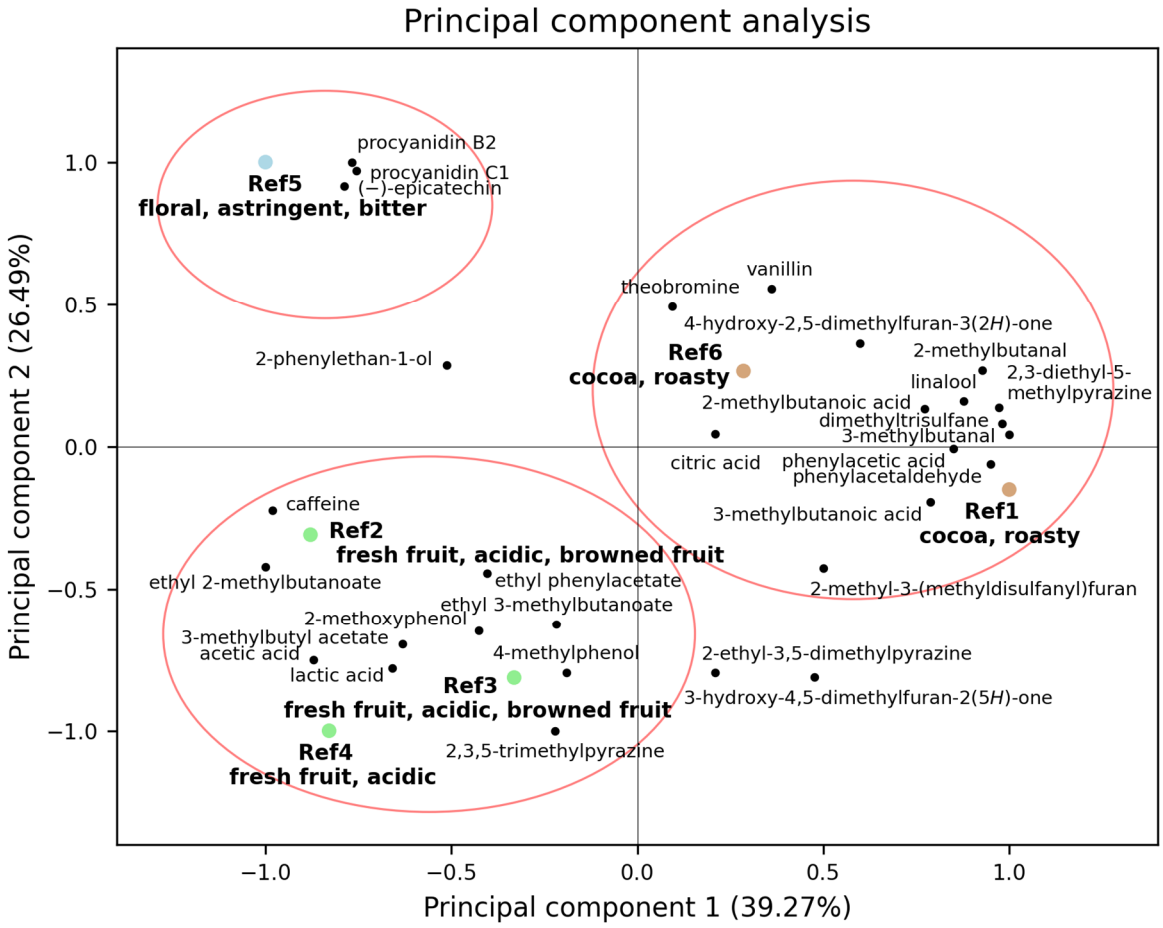

Figure 13: Principal component analysis applied to the DoT factors of flavor-active compounds with a DoT factor >1 in at least one of the chocolate samples[96]

The first cluster consisted of the three chocolates Ref2, Ref3, and Ref4, which were described as distinctly fruity and acidic. The three samples were associated with acetic acid in the PCA plot (cf. Figure 13) and the highest DoT factors of acetic acid were found in Ref2, Ref3 and Ref4 (Figure 14). Acetic acid exhibited very high olfactory DoT factors of >4000, which were higher than those of all other flavor-active compounds.[96] The olfactory DoT factors were calculated with an odor threshold of 350 in sunflower oil.[91] They might be overestimated because chocolate is not a pure fat matrix and acetic acid is partly present in the deprotonated form. The odor threshold of acetic acid in water is with 5600 much higher than in oil.[2] Nevertheless, the high values suggested an essential contribution of acetic acid to the intense acidity of Ref2, Ref3, and Ref4. In addition to its pungent impression and vinegar-like odor, acetic acid contributed to the sour taste. However, its taste threshold[43] is 343 times higher than its odor threshold.[91] Lactic acid, another taste-active compound, was associated with Ref2, Ref3, and Ref4 in the PCA plot and showed high gustatory DoT factors in the three chocolates (Figure 14). Therefore, we suggested an additional contribution of lactic acid to the perceived acidity of the samples. Even though the DoT factors of citric acid were not specifically high in this cluster,[96] the compound might have additionally influenced the acidic sensation of the samples through its sour taste. Finally, we assumed that the acidic flavor was mainly evoked by acetic acid in combination with lactic acid and citric acid.[96]

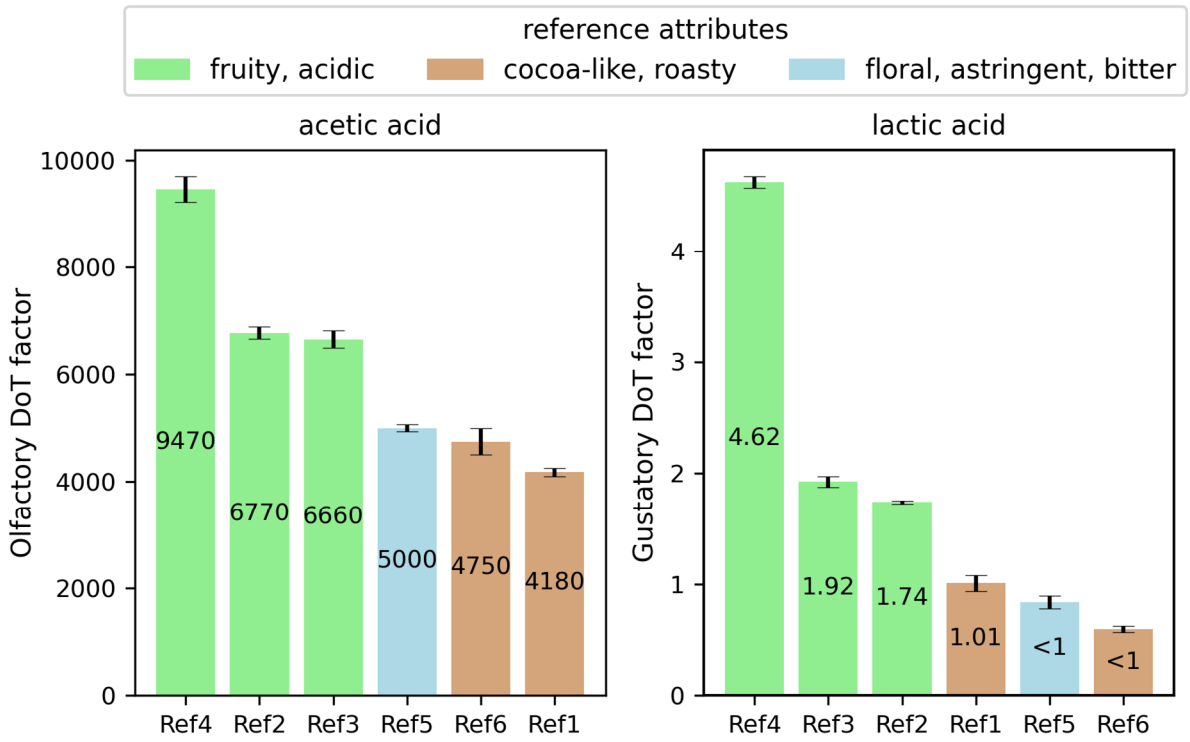

Figure 14: Olfactory DoT factors of acetic acid (left) and gustatory DoT factors of lactic acid (right) in the six reference chocolates (reference attributes according to Table 4)

Ref2, Ref3, and Ref4 were further referenced with distinct fruity odor notes and associated with high DoT factors of odor-active esters in the PCA plot (cf. Figure 13). The sum of the DoT factors of the fruity smelling esters ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and 3-methylbutyl acetate was clearly highest among all the chocolates for Ref2, Ref3, and Ref4

(Figure 15). In detail, the DoT factor of the banana-like smelling 3-methylbutyl acetate was highest in Ref4 (3.64) followed by Ref3 (3.02). The DoT factor of 3-methylbutyl acetate in Ref2 was <1, but this chocolate showed the highest DoT factor of ethyl 2-methylbutanoate (8.18). The highest DoT factor of ethyl 3-methylbutanoate (3.82) was found in Ref3. We suggested that the high DoT factors of the fruity smelling esters in the three samples corresponded to their distinct fruity odor notes.[96] We further assumed that none of the esters alone was responsible for the distinct fruity odor. Instead, it seemed to be the combination of all the fruity smelling esters that contributed to the distinct fruity odor of Ref2, Ref3, and Ref4 (Figure 15).

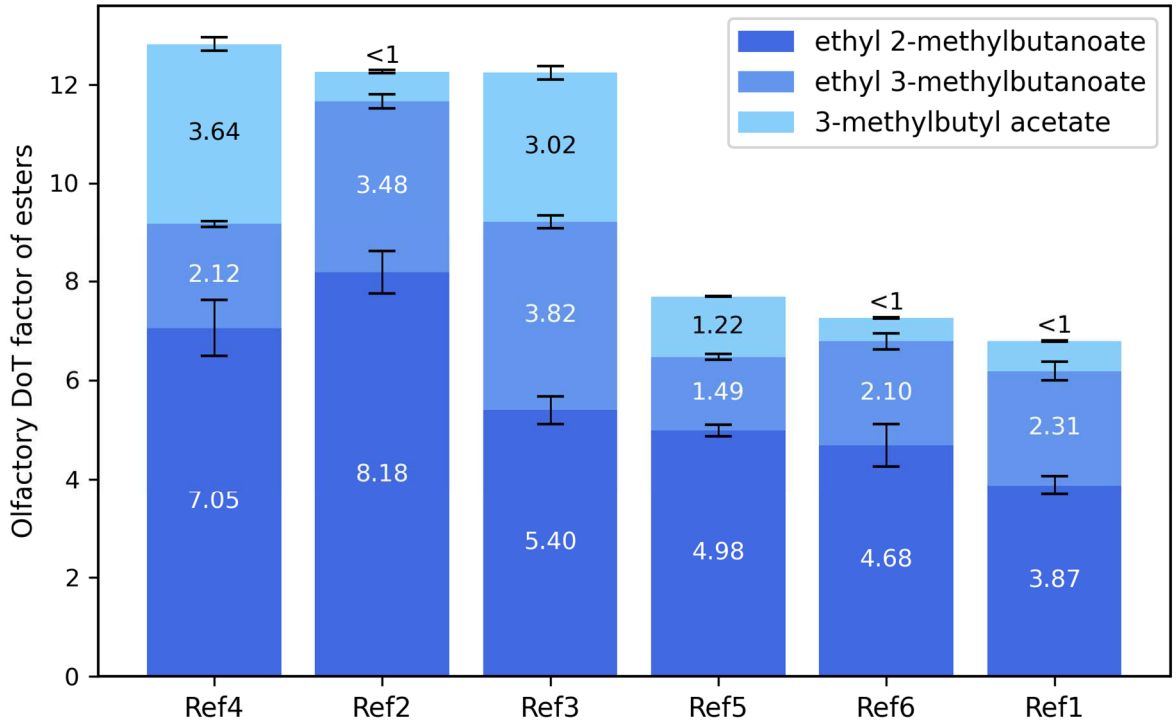

Figure 15: Olfactory DoT factors of the fruity smelling esters ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and 3-methylbutyl acetate in the six reference chocolates

Among several other compounds, ethyl 3-methylbutanoate and 3-methylbutyl acetate had been suggested by Rottiers et al.[79] to be responsible for the fruity odor notes in cocoa liquor. The important role of these two esters was confirmed by our data. However, we could not confirm the importance of the other compounds suggested by Rottiers et al.[79] for the fruity odor. For example, we identified linalool as an odor-active compound in the six reference chocolates, but there was no indication of an important contribution of linalool to the fruity odor. The olfactory DoT factor of linalool in Ref3 with 75.4 was the second highest of all samples, but Ref2 and Ref4 showed the lowest DoT factors of linalool (Figure 16).

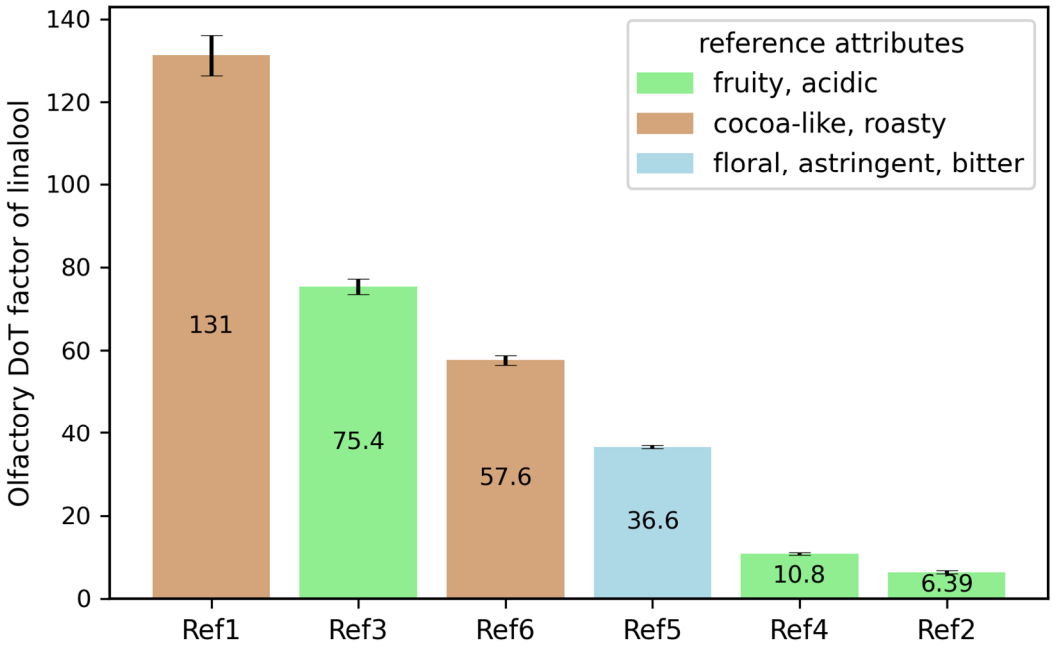

Figure 16: Olfactory DoT factors of linalool in the six reference chocolates (reference attributes according to Table 4)

While the distinct overall fruitiness of Ref2, Ref3, and Ref4 could be well explained by the high DoT factors of the fruity smelling esters, the background of the browned fruits note in Ref2 and Ref3 was more difficult to elucidate at the molecular level. Interestingly, clearly lower olfactory DoT factors of acetic acid were observed in Ref2 and Ref3 than in Ref4 (cf. Figure 14). The high DoT factor of acetic acid in Ref4 indicated a more intense acidic perception in Ref4 than in Ref2 and Ref3. Our data suggested that the acidic perception might have shifted the fruity odor perception in Ref4 towards a more intense impression of fresh fruits than of browned fruits. Vice versa, the browned fruit odor notes might have been perceived more intensely in Ref2 and Ref3 due to the lower DoT factors of acetic acid.[96]

Another cluster in the PCA plot was formed by Ref1 and Ref6 (cf. Figure 13). Both chocolates were described as distinctly cocoa-like and roasty. This cluster was associated with 13 flavor-active compounds. Ref1 and Ref6 showed high olfactory DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde (Figure 17). The high DoT factors of 3-methylbutanal agreed with the special importance of this compound for a distinct cocoa-like odor. In addition, high DoT factors of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane were found in Ref1 and Ref6 (Figure 18). All odorants that were linked to distinct cocoa-like and roasty odor notes according to our data have been previously identified as key odorants in roasted cocoa products.[45–47] Interestingly, none of the odorants were described as cocoa-like during AEDA. Therefore, we suggested that the combination of these odorants caused the distinct cocoa-like odor in the two samples.[96]

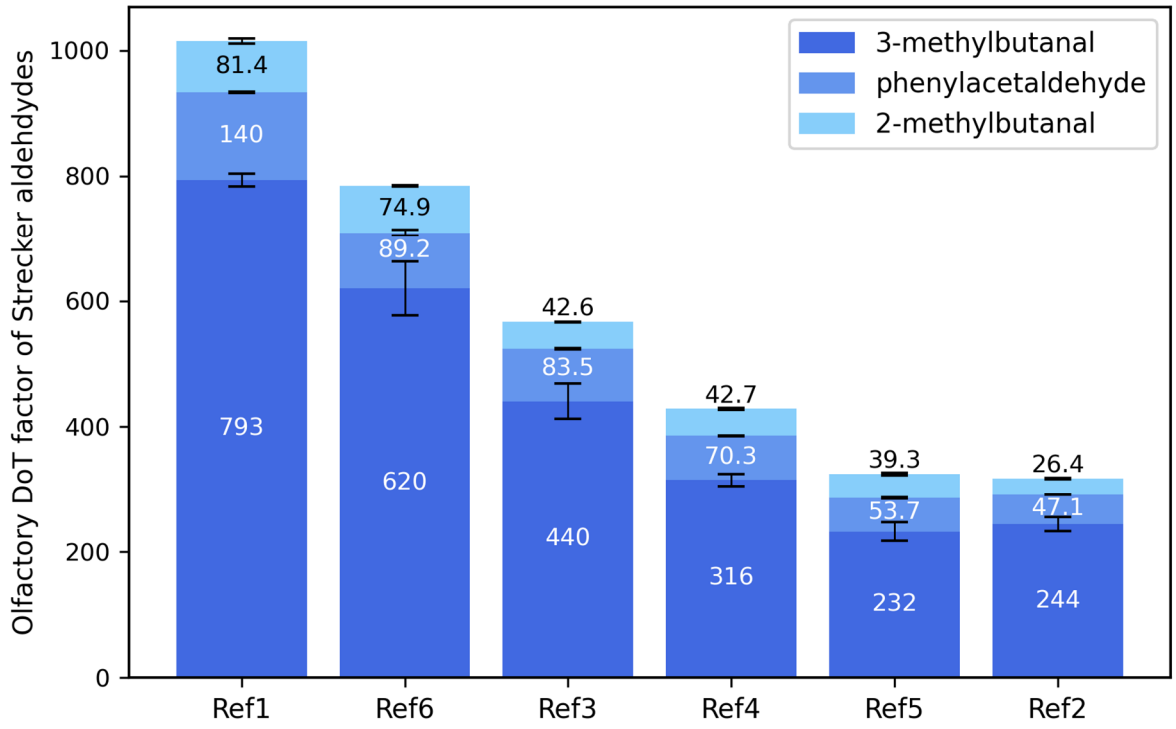

Figure 17: Olfactory DoT factors of the Strecker aldehydes in the six reference chocolates

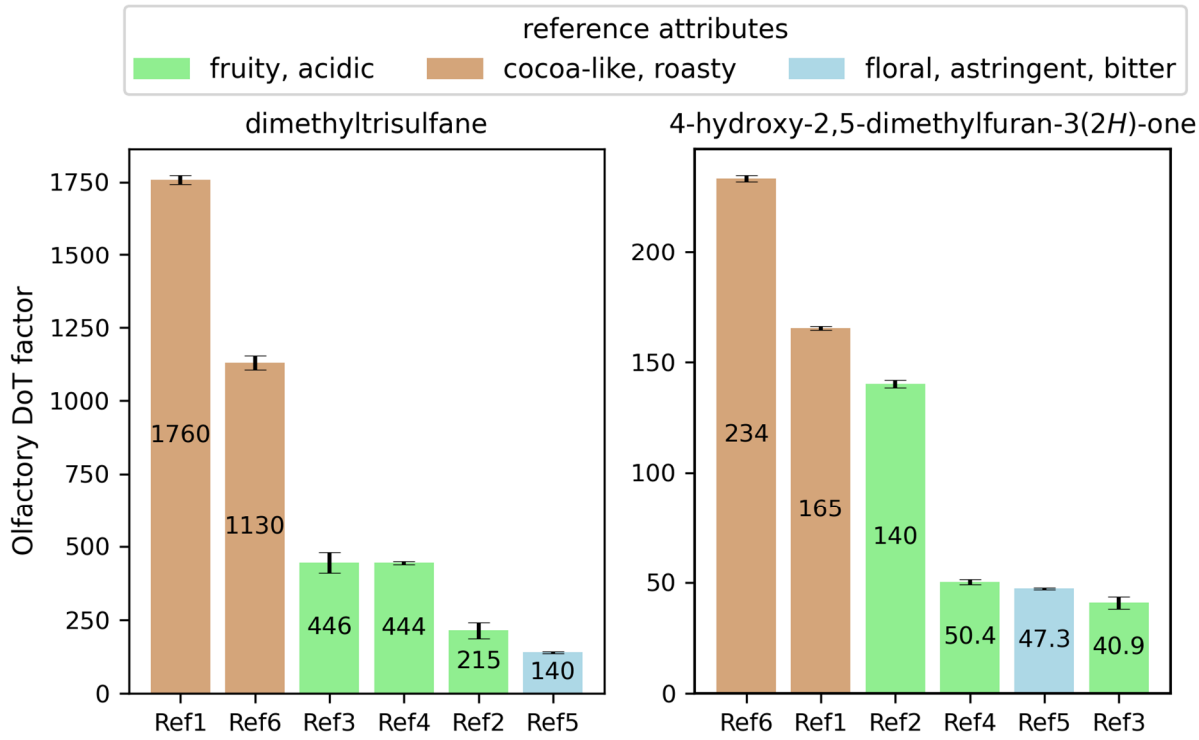

Figure 18: Olfactory DoT factors of dimethyltrisulfane (left) and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one (right) in the six reference chocolates (reference attributes according to Table 4)

Ref5 was clearly separated from the two clusters in the PCA plot (cf. Figure 13) and was referenced as distinctly floral, astringent, and bitter. We assumed that the floral odor of Ref5 was mainly caused by 2-phenylethan-1-ol.[96] With 17.7, this floral smelling compound showed the highest DoT factor in Ref5 (Figure 19). The important role of 2-phenylethan-1-ol for a floral

odor in cocoa and chocolate was previously suggested[76,77,79,80,98] and confirmed by our data. However, in another study, linalool was assumed to be mainly responsible for floral notes in chocolate.[75] This was not confirmed by us. The DoT factor of linalool in Ref5 with 36.6 was not especially high when compared to those in the other chocolates (cf. Figure 16). Floral and honey-like odor notes in Nacional samples were additionally ascribed to high concentrations of phenylacetaldehyde, linalool, 2-phenylethyl acetate, and ethyl phenylacetate when compared to CCN51 samples.[76] The olfactory DoT factors of 2-phenylethyl acetate and ethyl phenylacetate were <1 in Ref5. The olfactory DoT factors of the other floral smelling odorants, namely phenylacetaldehyde and phenylacetic acid, were not particularly high in Ref5 (Figure 20). The DoT factors of phenylacetaldehyde, phenylacetic acid, and linalool were even higher in Ref1 and Ref6 (cocoa, roasty) although these chocolates were not described as distinctly floral.[96]

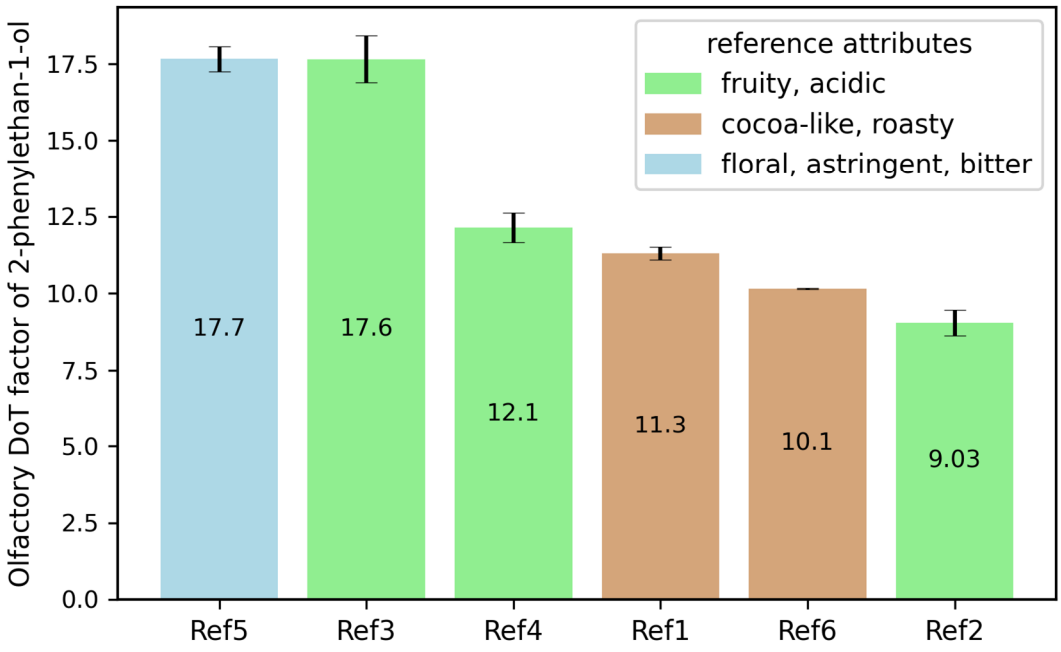

Figure 19: Olfactory DoT factors of 2-phenylethan-1-ol in the six reference chocolates (reference attributes according to Table 4)

Ref5 was additionally associated with polyphenols in the PCA plot (cf. Figure 13). Ref5 showed by far the highest DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 (Figure 21). Therefore, we assumed that these compounds were mainly responsible for the distinct perception of astringency in this sample. According to the DoT concept and previous findings, theobromine has the greatest influence on the bitter taste.[43,96] Ref5 showed the second highest DoT factor of 78.6 for theobromine among all samples (Figure 22). However, especially (–)-epicatechin is known to enhance bitter perception.[43] Therefore, we assumed that the intense bitter taste in Ref5 was caused by high DoT factors of theobromine in combination with high DoT factors of polyphenols such as (–)-epicatechin.[96]

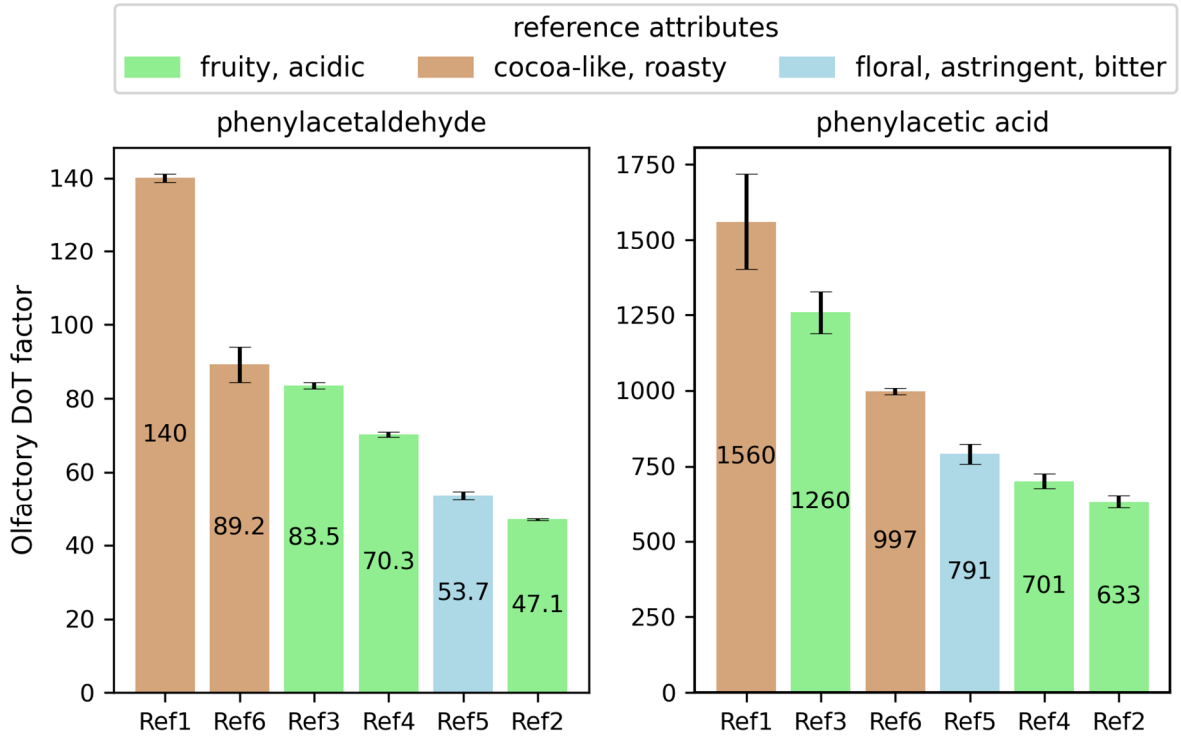

Figure 20: Olfactory DoT factors of phenylacetaldehyde (left) and phenylacetic acid (right) in the six reference chocolates (reference attributes according to Table 4)

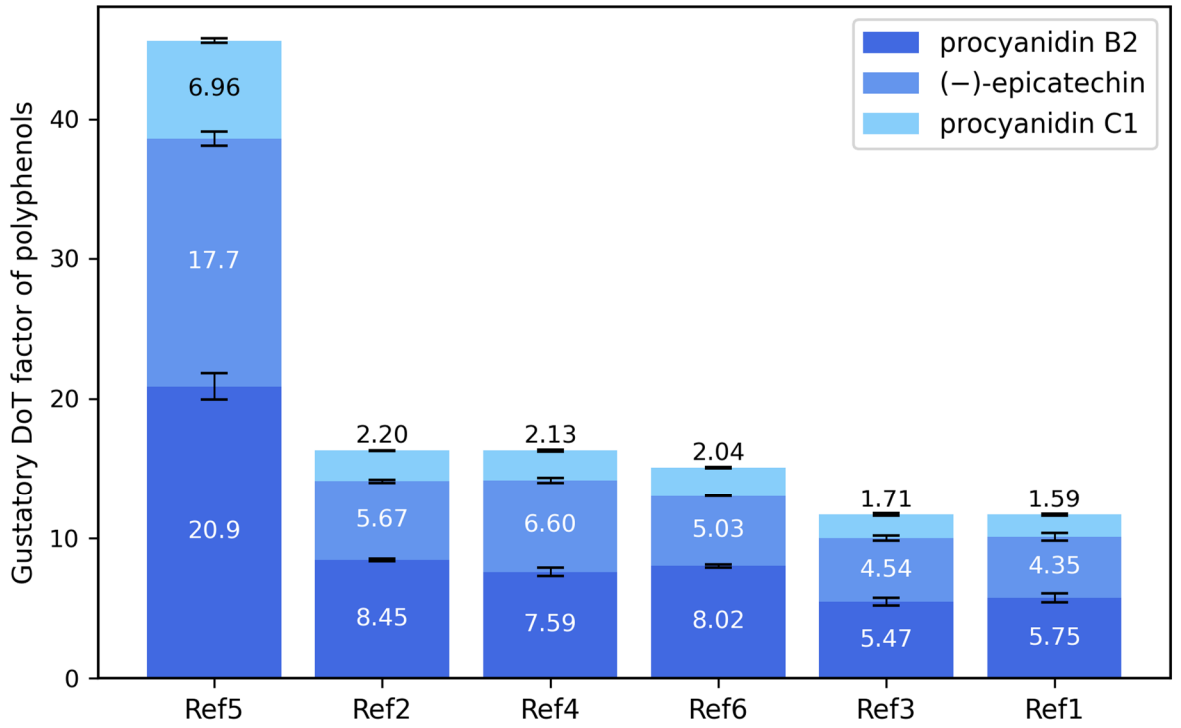

Figure 21: Gustatory DoT factors of the polyphenols in the six reference chocolates

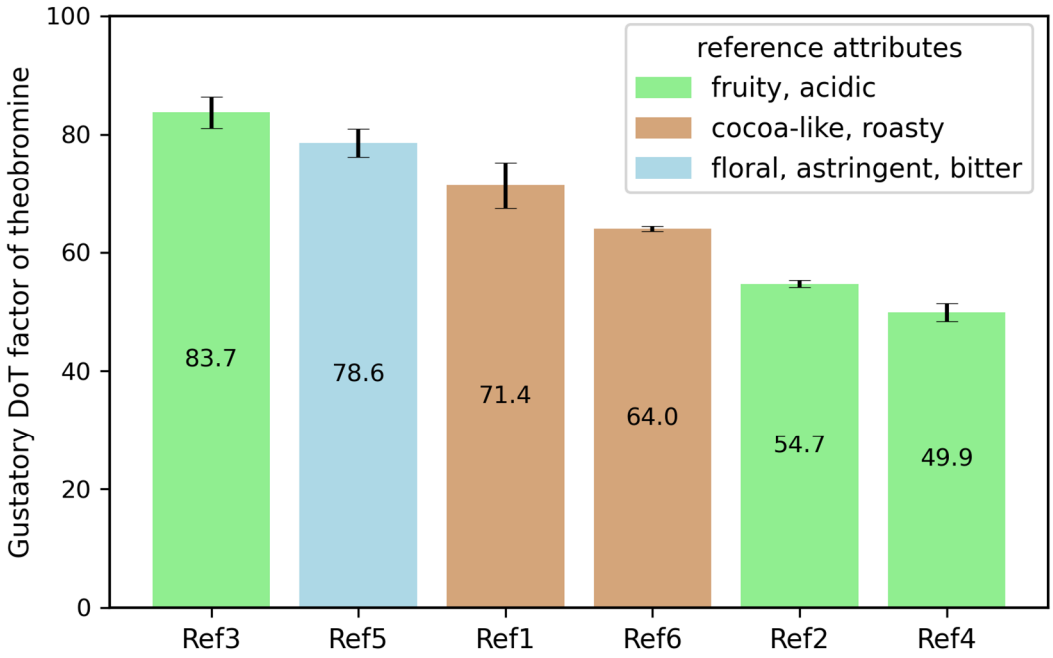

Figure 22: Gustatory DoT factors of theobromine in the six reference chocolates (reference attributes according to Table 4)

In summary, we have shown for the first time how specific and distinct flavor characteristics of dark chocolates are reflected in their flavor compound compositions.[96] We were able to suggest flavor-active compounds being responsible for the different flavor properties of the sensory reference samples. Fruity and acidic flavor notes were associated with high DoT factors of acetic acid and fruity smelling esters such as ethyl 2-methylbutanaote, ethyl 3-methylbutanoate, and 3-methylbutyl acetate. The DoT factor of acetic acid might have additionally influenced the fruity perception in terms of the intensity of fresh fruit and browned fruit notes. We suggested high DoT factors of the cocoa key odorants 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyl-trisulfane as indicators for flavor profiles dominated by distinct cocoa-like and roasty flavor notes. We linked a distinct floral odor predominantly to a high DoT factor of 2-phenylethan-1-ol. Finally, intense astringent and bitter perceptions were associated with high DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 in combination with a high DoT factor of theobromine.

6.3 Impact of the cocoa bean variety on the flavor compound compositions of dark chocolates

Bean-to-bar products are typically made from fine flavor cocoa of defined variety and origin. Such chocolates often also have unique flavor profiles with fine flavor properties such as the ones decoded in section 6.2. Variety is one criterion among others for classifying cocoa as fine flavor cocoa. However, the impact of cocoa bean variety on the flavor compound compositions of dark chocolates has not been comprehensively studied. To investigate this, we compared the flavor compound compositions of 16 single variety dark chocolates.[99] The sample set consisted of six Criollo chocolates, six Trinitario chocolates, three Forastero chocolates, and one Nacional chocolate. The chocolates are listed in Table 7. Chocolates Ref1–Ref6 have already been discussed in section 6.2. The other ten chocolates were commercial small batch chocolates. The flavor-active compounds selected for analysis were the same as those quantitated in section 6.2. The obtained concentrations of the 27 odorants and 8 tastants were normalized to the cocoa content for better comparability between the samples. The DoT factors were then calculated from the normalized concentrations. The DoT factors of ethyl cinnamate, 2-phenylethyl acetate, γ-nonalactone, γ-decalactone, and cyclo(L-pro-L-val) were <1 in all samples. Therefore, these compounds were considered of little relevance to the overall flavor properties and thus, were not included in the data evaluation. In addition, the Nacional sample was excluded from the statistical data analysis. This sample (Ref5) will be discussed separately.

Table 7: Metadata of the 16 single variety dark chocolates

sample code[a]	bean variety[b]	cocoa percentage[c]
Ref1	Forastero	75
Ref2	Criollo	75
Ref3	Trinitario	75
Ref4	Trinitario	75
Ref5	Nacional	75
Ref6	Forastero	75
SB11	Trinitario	70
SB12	Trinitario	70
SB7	Criollo	70
SB10	Criollo	70
SB17	Trinitario	70
SB19	Trinitario	72
SB4	Criollo	70
SB6	Criollo	80
SB27	Criollo	75
SB28	Forastero	100

[a]Samples SB were part of a bigger sample set.[82] [b]Cocoa bean variety according to product labelling. [c]Cocoa percentage as declared on the packaging

To get a first overview, the DoT factors were subjected to PCA (Figure 23) and visualized in a heat map (Figure 24).[99] Overall, the PCA plot and the heat map indicated similarities in the flavor compound compositions of chocolates made from the same variety, especially for the three Forastero chocolates and the three Trinitario chocolates Ref3, Ref4, and SB19.

However, we also observed differences in chocolates made from the same variety. Our data suggested that the flavor compound compositions varied more in the Trinitario and Criollo chocolates than in the Forastero chocolates.

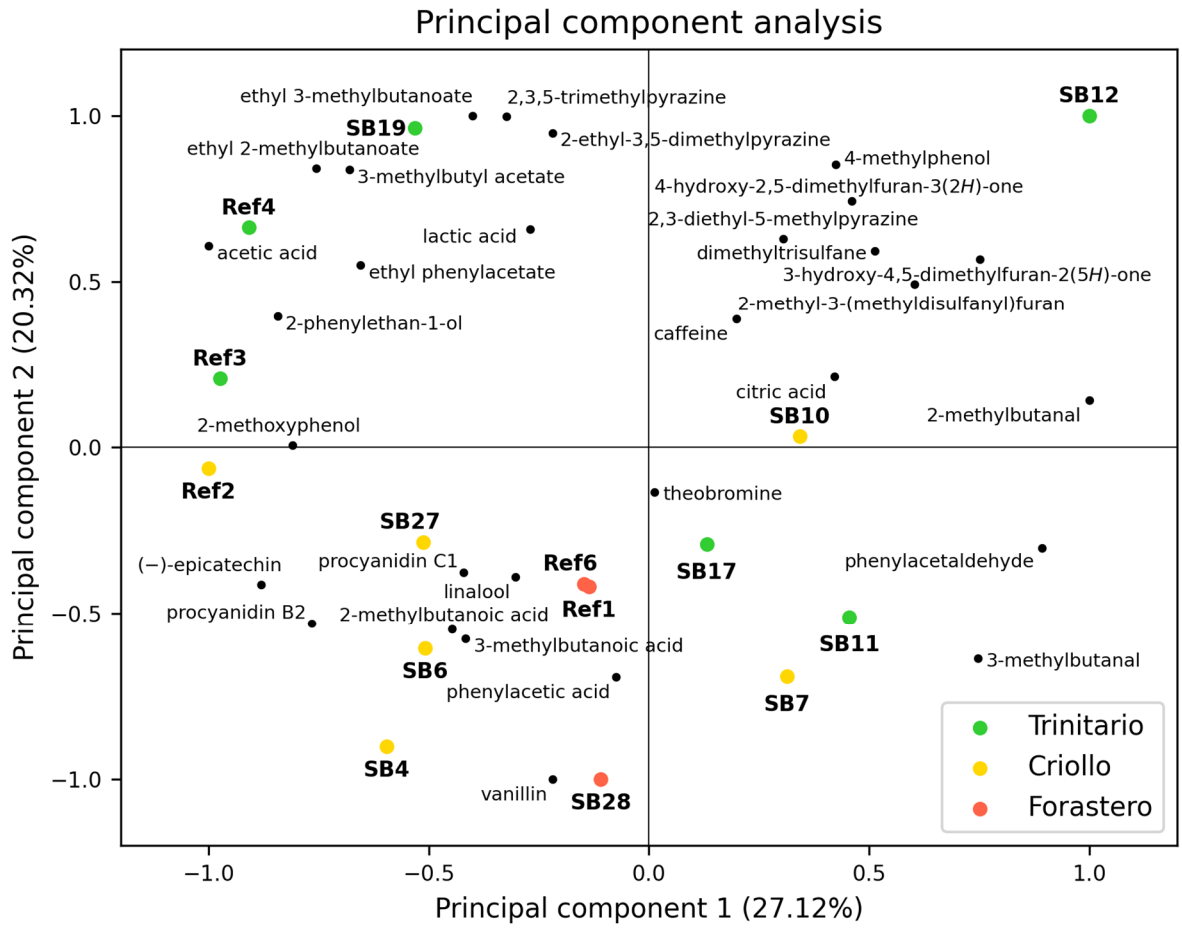

Figure 23: Principal component analysis applied to the DoT factors of flavor-active compounds with a DoT factor of >1 in at least one of the single variety dark chocolate samples (Ref5 excluded)[99]

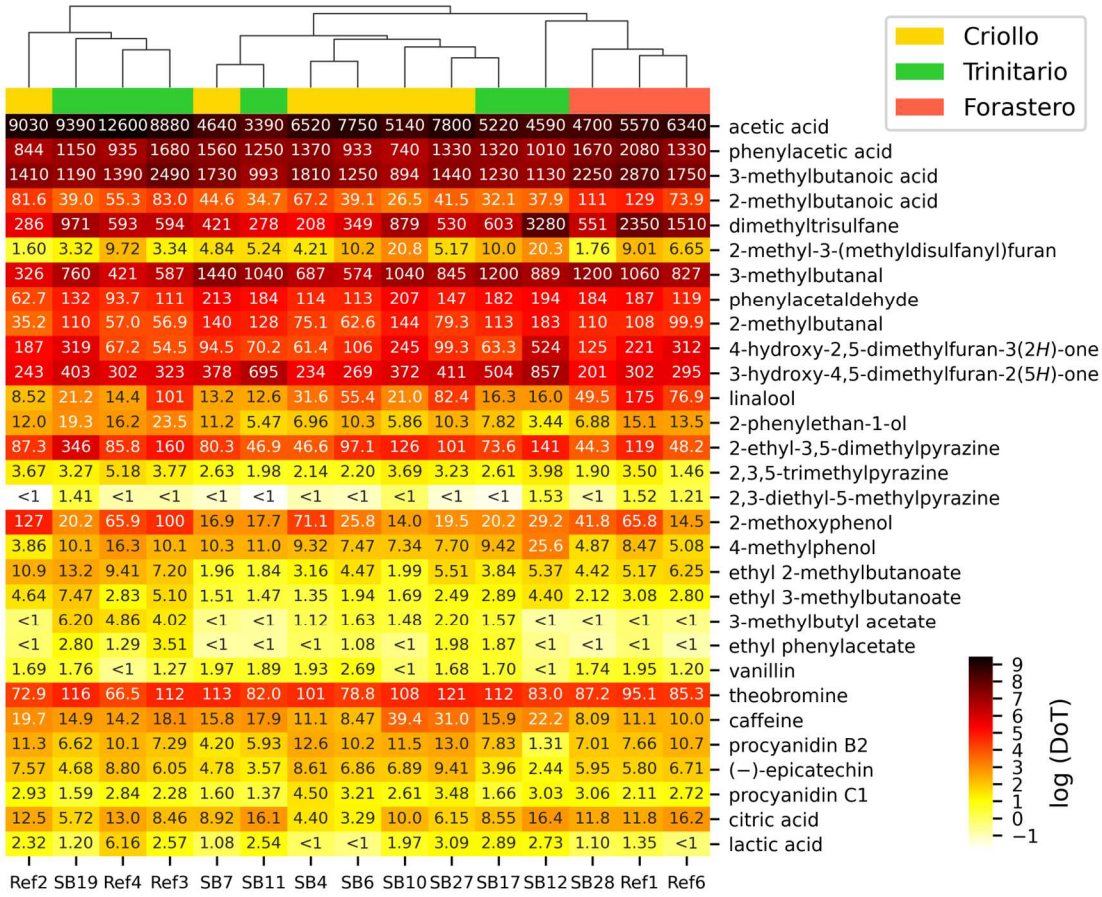

Figure 24: Heat map showing the DoT factors of flavor-active compounds with a DoT factor of >1 in at least one of the single variety dark chocolates samples (Ref5 excluded)[99]

6.3.1 Flavor-active compounds in the Forastero chocolates

The heat map and the PCA plot indicated similarities in the flavor compound compositions of the three Forastero chocolates SB28, Ref1, and Ref6. In particular, Ref1 and Ref6 were linked closely together. The low values on principal component (PC) 2 were associated with high olfactory DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde. Ref1 and Ref6 were both referenced with distinct cocoa-like and roasty flavor notes (cf. Table 4) and especially 2- and 3-methylbutanal were shown to be characteristic of cocoa-like flavor notes (cf. section 6.2). The Forastero chocolates showed high olfactory DoT factors of other compounds characteristic of cocoa-like flavor notes, such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane (cf. Figure 24). These findings were consistent with the reported strong chocolate-like flavor of Forastero beans after fermentation and roasting.[21] All three Forastero chocolates had high DoT factors of these compounds in common. Interestingly, many of these compounds were found with even higher DoT factors in chocolates made from other varieties. SB7 (Criollo) showed higher DoT factors of the three Strecker aldehydes (Figure 25) than the Forastero chocolates, and the DoT factors of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane were clearly higher in SB12 (Trinitario) than in the Forastero chocolates. Consequently, high DoT factors of these compounds were not exclusive to the Forastero chocolates.[99]

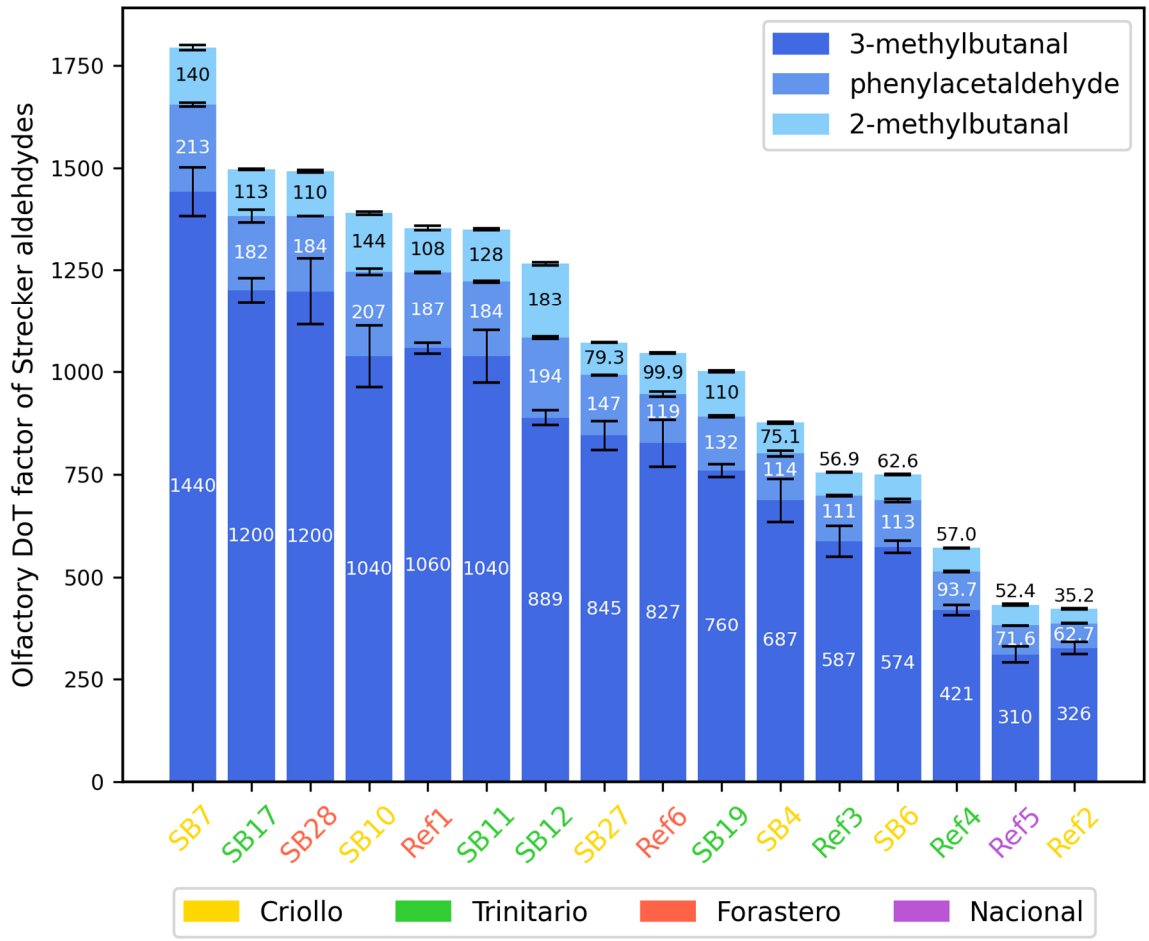

Figure 25: Olfactory DoT factors of the Strecker aldehydes in the single variety chocolates

The Forastero chocolates further had high olfactory DoT factors of phenylacetic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, and linalool in common. These compounds showed significantly higher mean values in the Forastero chocolates when the DoT factors within the varieties were averaged (Figure 26). Previous studies found high linalool concentrations primarily in Nacional and Criollo cocoa and low concentrations in Forastero cocoa.[75,83,100,101] The high olfactory DoT factors in the Forastero chocolates were contradictory to these findings. However, Frauendorfer and Schieberle[47] also found higher linalool concentrations in Forastero cocoa beans than in Criollo cocoa beans. In addition, Tuenter et al.[84] could not confirm high linalool concentrations in Nacional chocolate when compared to a West African blend chocolate. In fact, in the corresponding West African blend liquor the linalool concentration was significantly higher.[84] In another study, linalool was even identified as a characteristic compound of West African dark chocolates.[87] These findings are consistent with our results, as the three Forastero chocolates analyzed were all made with cocoa beans from West Africa.

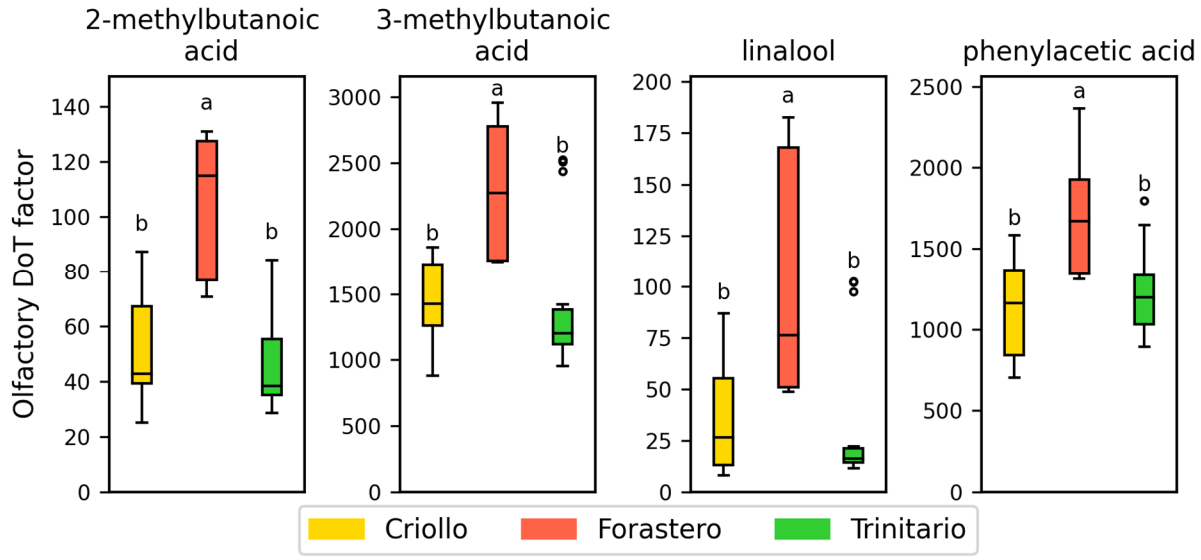

Figure 26: Box plots indicating the olfactory DoT factors of compounds that showed significantly higher DoT factors in the Forastero chocolates

The olfactory DoT factors of pyrazines were not remarkably high in the Forastero chocolates (cf. Figure 24). Higher DoT factors of pyrazines in the Forastero chocolates were expected due to the longer fermentation times and higher roasting temperatures typically applied to Forastero beans.[24,33,102] Counet et al.[85] found predominantly higher pyrazine concentrations in two Forastero liquors compared to liquors from other varieties. Tuenter et al.[84] reported higher pyrazine concentrations in liquor and chocolate from a West African blend than in the ones from Nacional cocoa beans. Our data did not confirm their findings but were consistent with those of Frauendorfer and Schieberle[46,47] who did not observe higher pyrazine concentrations in Forastero cocoa beans than in Criollo cocoa beans.

The concentrations of the two lactones γ-decalactone and γ-nonalactone were significantly highest in the Forastero chocolates (Figure 27), but their olfactory DoT factors were <1 in all samples. Nevertheless, the higher concentrations in the Forastero chocolates might have impacted the overall flavor through additive effects together with other lactones, that were not quantitated. Similar to our observation, Frauendorfer and Schieberle[47] found higher concentrations of a δ-octenolactone in Forastero cocoa beans than in Criollo cocoa beans. Recently, the coconut-like smelling (*R*)-δ-2-decenolactone was identified as the lactone with the highest flavor impact in samples with pronounced coconut odors.[62] Further studies are needed to clarify whether this lactone shows high concentrations in Forastero chocolates, similar to γ-decalactone and γ-nonalactone.

Not only the odor-active compounds, but also the taste-active compounds showed clear similarities within the group of Forastero chocolates. The gustatory DoT factors of caffeine were lowest in the Forastero chocolates which was in line with other studies.[84] Theobromine was assumed to have the highest impact on bitter perception according to the DoT concept. The gustatory DoT factors of this compound did not differ significantly between varieties. The DoT factors of the bitter tasting cyclo(L-pro-L-val) were <1 in all samples and the concentrations were not particularly high in the Forastero chocolates.[99] However, a recent investigation with 33 chocolates showed that Forastero chocolates were characterized by high concentrations of 2,5-diketopiperazines including cyclo(L-pro-L-val).[82]

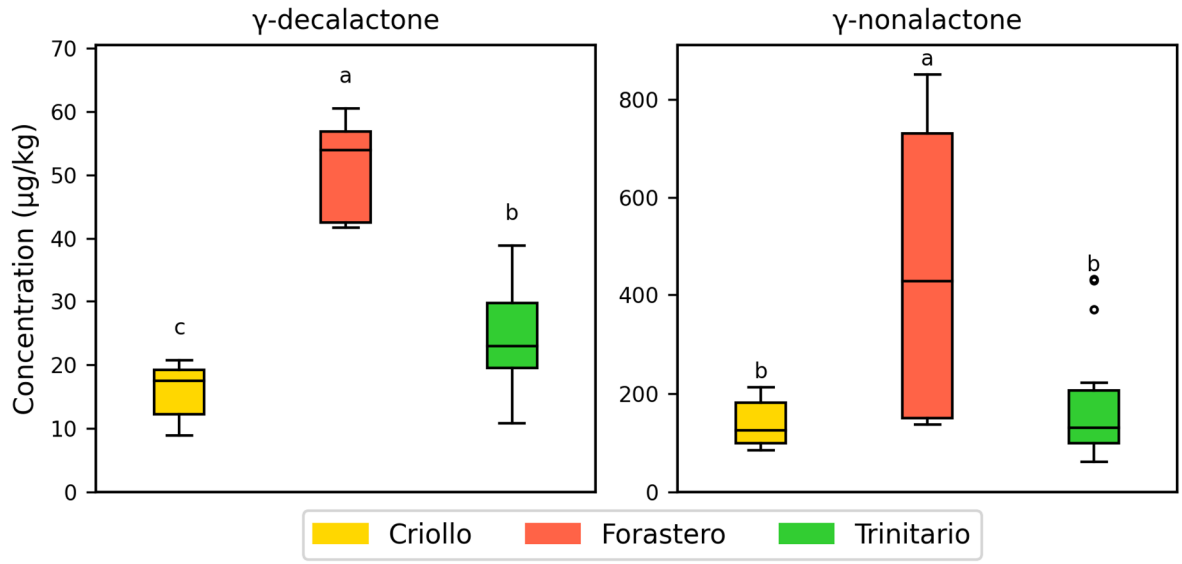

Figure 27: Box plots showing the concentrations of γ-decalactone and γ-nonalactone

In summary, the Forastero chocolates shared common characteristics in their flavor compound compositions, such as high DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde, as well as high DoT factors of 2- and 3-methylbutanoic acid, phenylacetic acid, and linalool. The DoT factors of caffeine and esters were low in the Forastero chocolates.[99]

6.3.2 Flavor-active compounds in the Trinitario chocolates

The heat map (cf. Figure 24) and the PCA plot (cf. Figure 23) indicated a higher variability in the flavor compound compositions among the Trinitario chocolates than among the Forastero chocolates.[99] Nevertheless, some similarities were observed within the Trinitario group. In particular, the three chocolates Ref3, Ref4, and SB19 shared common characteristics and were linked closely together in the PCA plot and the heat map. Their low values of PC 1 and high values of PC 2 in the PCA plot were associated with high olfactory DoT factors of esters and acetic acid. Ref3, Ref4, and SB19 showed high DoT factors for acetic acid (Figure 28) and the esters ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, and ethyl phenylacetate (Figure 29). These compounds were associated with a flavor profile dominated by fruity and acidic notes, and Ref3 and Ref4 were referenced as distinctly fruity and acidic (cf. Table 4 in section 6.2). SB19 showed even higher DoT factors of the fruity smelling esters ethyl 2-methylbutanoate (13.2), ethyl 3-methylbutanoate (7.47), and 3-methylbutyl acetate (6.20) than Ref3 and Ref4. The DoT factor of acetic acid in SB19 was 9390, which was the second highest among all samples. Trinitario products are known for their fruity and acidic sensory characteristics and high concentrations of acetic acid were found in other studies.[74,80,83] Consequently, the three Trinitario chocolates Ref3, Ref4, and SB19 showed the expected flavor compound profiles. Quin et al.[83] made a similar observation and found higher concentrations of acetic acid and esters in Trinitario cocoa than in Criollo and Forastero cocoas.

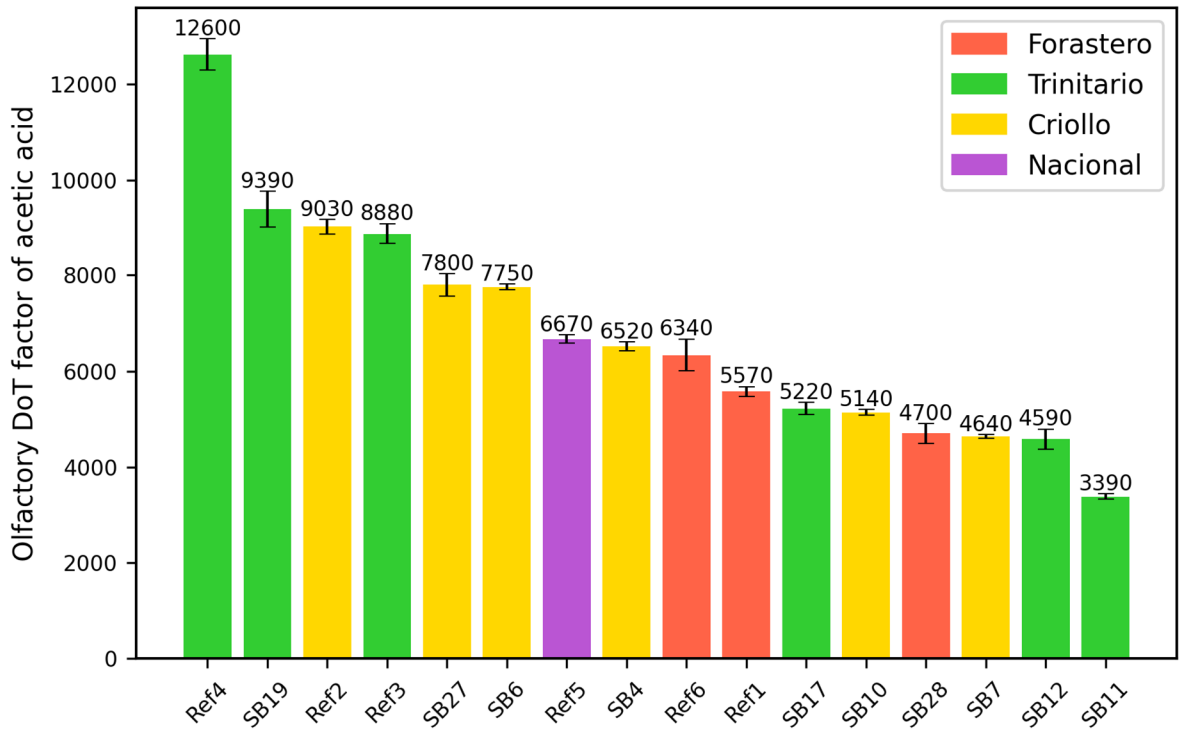

Figure 28: Olfactory DoT factors of acetic acid in the single variety dark chocolates

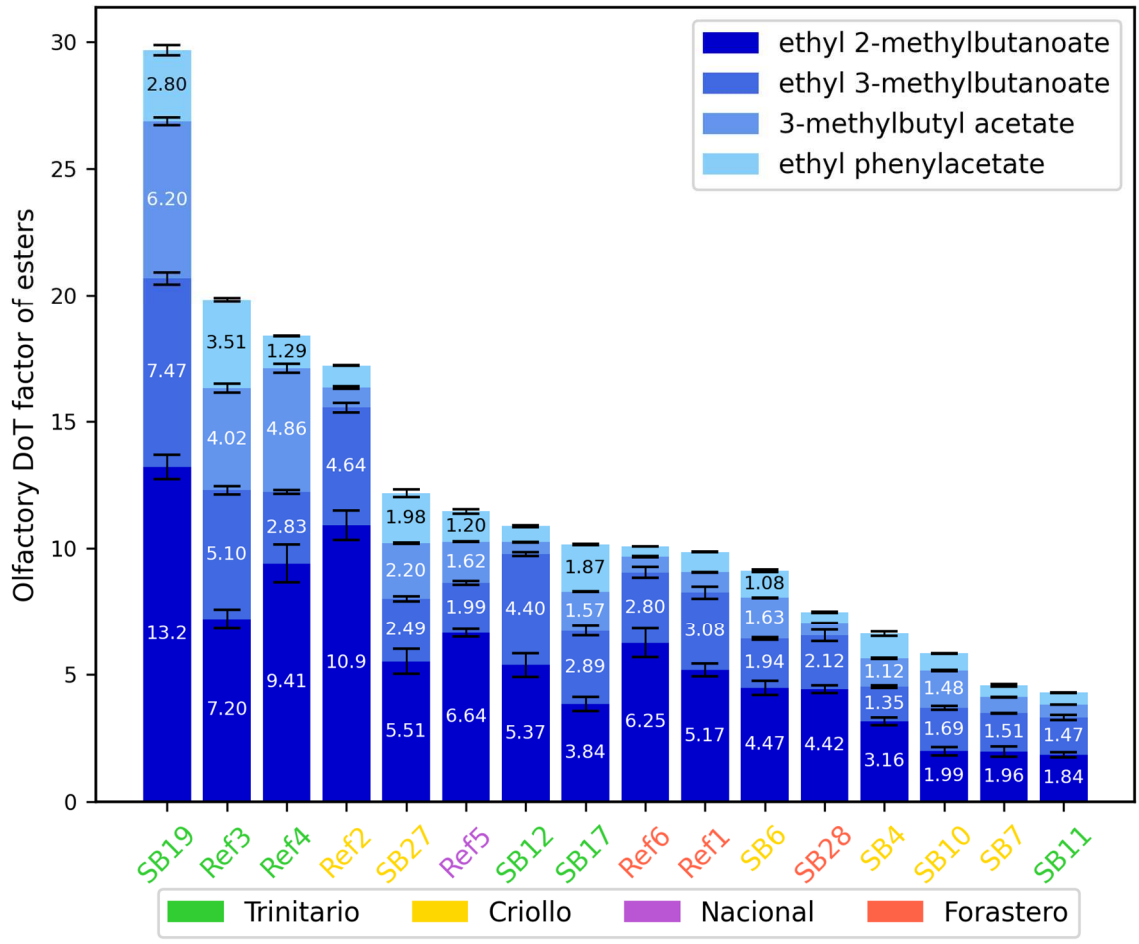

Figure 29: Olfactory DoT factors of esters in the single variety dark chocolates (numerical values are shown for DoT factors >1)

The Trinitario chocolates SB17, SB11, and SB12 were not clustered with Ref3, Ref4, and SB19 in the heat map and the PCA plot. The three chocolates showed low DoT factors of acetic acid (Figure 28). However, the sum of esters in SB12 and SB17 was higher than in all Forastero chocolates and most Criollo chocolates (Figure 29). Thus, high DoT factors of fruity smelling esters were found in most Trinitario chocolates. Interestingly, the lowest DoT factors of esters and acetic acid were observed in SB11. Consequently, it must be noted that the variety does not guarantee a specific flavor profile.[99]

SB17 and SB11 were closely linked to the three Forastero chocolates in the PCA plot. Both chocolates showed similar characteristics as the Forastero chocolates, such as high DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde, which were in the range of those determined in the Forastero chocolates (cf. Figure 25). The Trinitario sample SB12 was clearly separated from all other chocolates in the PCA plot and linked to the three Forastero chocolates in the heat map. SB12 showed high DoT factors of most compounds indicative of intense cocoa-like flavor notes, such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, dimethyltrisulfane, and 2-methylbutanal (cf. Figure 24). The DoT factors of these compounds were even higher than the corresponding values in the Forastero chocolates. Furthermore, SB12 showed the highest olfactory DoT factors of 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-methylphenol, and 2,3-diethyl-5-methylpyrazine among all samples.[99]

In summary, the Trinitario chocolates shared common features in their flavor compound compositions, such as high DoT factors of fruity smelling esters and acetic acid. However, high DoT factors of these compounds were not observed in all Trinitario chocolates. In addition, some of the Trinitario chocolates showed similar characteristics to the three Forastero chocolates. The high variability in the flavor compound compositions of the Trinitario chocolates demonstrated that not all chocolates from one variety show similar flavor profiles and the variety alone is not a clear determinant of flavor quality.[99] The high variability in the flavor compound compositions in the Trinitario chocolates could result from the fact that Trinitario is a hybrid between Criollo and Forastero.[21] The flavor compound composition might vary depending on whether the Criollo or the Forastero background is dominant, as has been suggested for 2,5-diketopiperazine concentrations in single variety dark chocolates.[82]

6.3.3 Flavor-active compounds in the Criollo chocolates

The heat map (cf. Figure 24) indicated a high variability of the Criollo samples in their flavor compound compositions which, according to the PCA plot (cf. Figure 23), was less pronounced than that of the Trinitario samples.[99] Ref2 (Criollo) was closely linked to the three Trinitario chocolates Ref3, Ref4, and SB19 in the heat map and the PCA plot. Like the three Trinitario chocolates, Ref2 showed high DoT factors of compounds associated with fruity and acidic flavor notes, such as ethyl 2-methylbutanoate, ethyl 3-methylbutanoate (cf. Figure 29), and acetic acid (cf. Figure 28), and was referenced as fruity and acidic (cf. Table 4 in section 6.2). SB27 also showed high DoT factors of esters and acetic acid (cf. Figure 28 and Figure 29). Consequently, high DoT factors of fruity smelling esters and acetic acid were not exclusive characteristics of Trinitario chocolates. In contrast to Ref2 and SB27, the two Criollo chocolates SB7 and SB10 showed low DoT factors of esters and acetic acid. They additionally showed

similar features as the three Forastero chocolates e.g., high DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde. SB7 showed the highest DoT factors of 3-methylbutanal and phenylacetaldehyde among all samples (cf. Figure 25).

The Criollo chocolates SB4 and SB6 did not show remarkably high or low DoT factors of specific compounds. Therefore, we assumed that the unique flavor profiles of the Criollo chocolates were caused by a specific combination of odorants rather than high DoT factors of individual odorants.[99] Low olfactory DoT factors of dimethyltrisulfane and low gustatory DoT factors of citric acid were characteristic of all Criollo chocolates and common features were observed in the DoT factors of the polyphenols. Except for SB7, the DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 were higher in most of the Criollo chocolates than in the Forastero and Trinitario chocolates (Figure 30). This observation was in line with the study of Counet et al., who observed high concentrations of procyanidins in two Criollo liquors and linked them to the short fermentation time of the Criollo beans.[85]

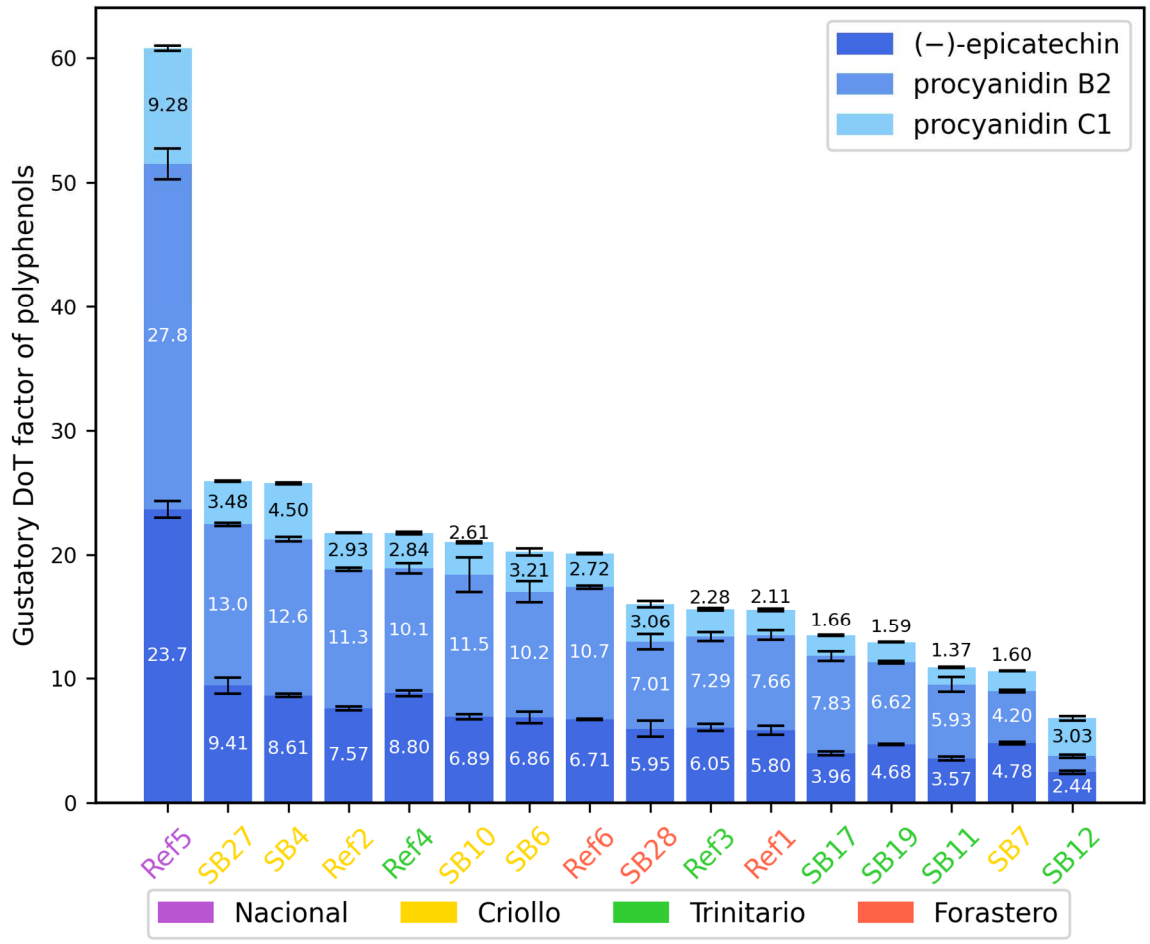

Figure 30: Gustatory DoT factors of polyphenols in the single variety dark chocolates

6.3.4 Flavor-active compounds in the Nacional chocolate

The sample set contained one Nacional chocolate (Ref5). Ref5 was characterized by very high DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 (cf. Figure 30) which is in line with previous reports on Nacional cocoa in the literature.[30,84] As discussed in section 6.2,

the distinct floral note in Ref5 was mainly associated with the high olfactory DoT factor of 2-phenylethan-1-ol in this sample. The DoT factor of 23.5 in Ref5 was outstanding even in the broader sample set of 16 chocolates (Figure 31).[99] Previous studies[76,84] reported high concentrations of 2-phenylethan-1-ol in Nacional samples, and floral notes are considered characteristic of Nacional cocoa.[3] The Nacional chocolate Ref5 showed low DoT factors of Strecker aldehydes, especially 3-methylbutanal (cf. Figure 25). Furthermore, the olfactory DoT factors of dimethyltrisulfane, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 2-methyl-3-(methyldisulfanyl)furan, and 2-ethyl-3,5-dimethylpyrazine in Ref5 were the lowest among all samples.[99] This is consistent with previous reports in the literature. Tuenter et al.[19] observed significantly lower concentrations of 2-methylbutanal, 3-methylbutanal, and pyrazines in a Nacional liquor than in a West African blend liquor. In contrast to the high linalool concentrations in Nacional cocoa reported in the literature,[75] with 48.8 the DoT factor of linalool in Ref5 was not especially high. In fact, all Forastero chocolates showed higher DoT factors of linalool (Figure 32). However, further Nacional samples need to be analyzed to further clarify the role of linalool in Nacional chocolate.

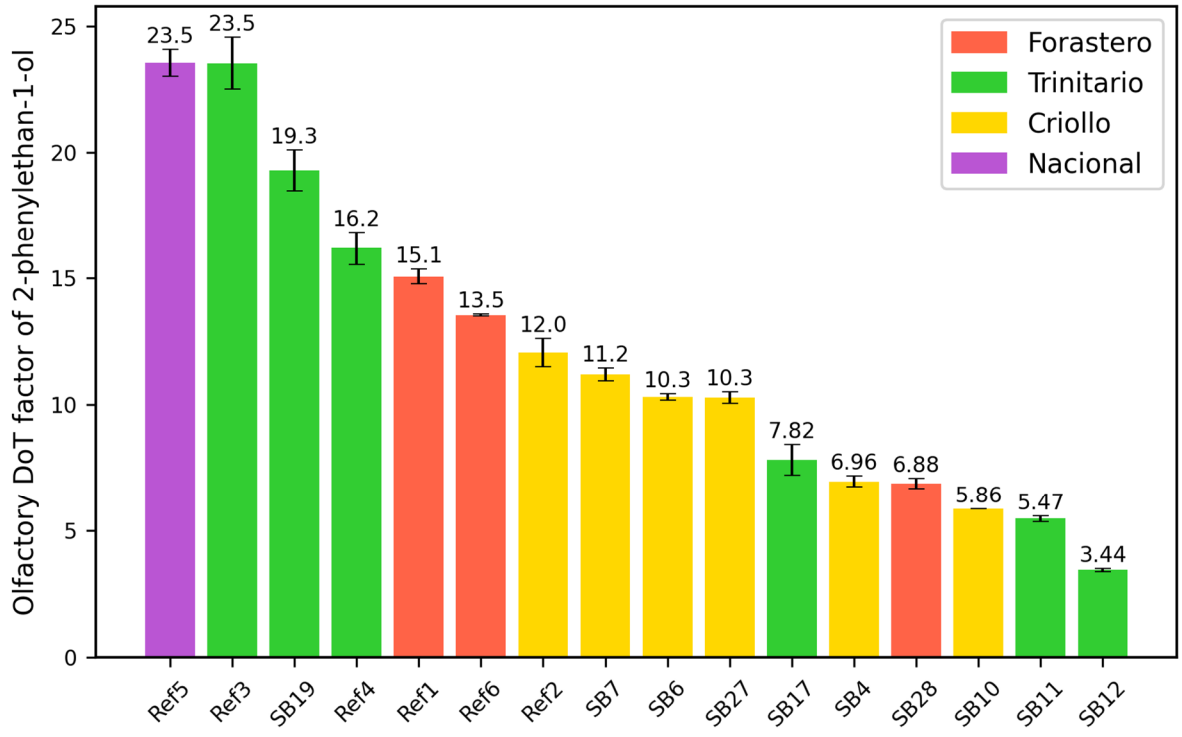

Figure 31: Olfactory DoT factors of 2-phenylethan-1-ol in the single variety dark chocolates

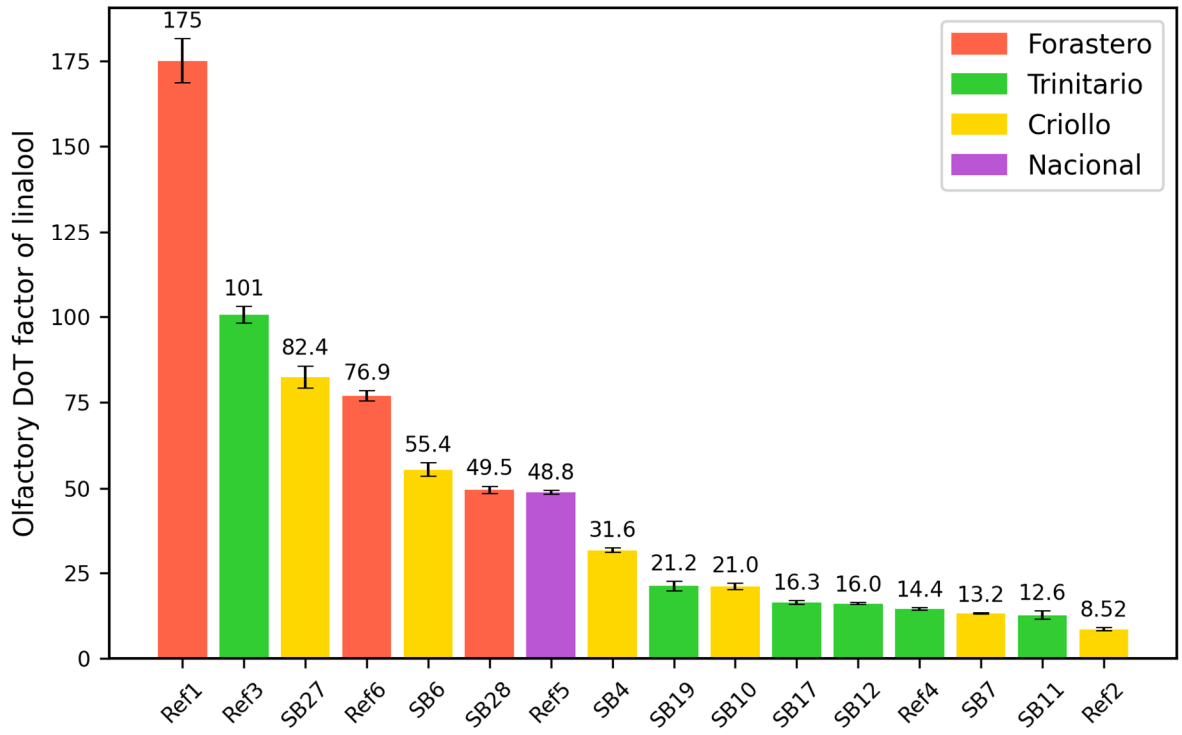

Figure 32: Olfactory DoT factors of linalool in the single variety dark chocolates

6.3.5 Conclusions

Common characteristics were observed for chocolates made from the same variety.[99] The three Forastero chocolates were characterized by high DoT factors of compounds such as 2-and 3-methylbutanal, 2- and 3-methylbutanoic acid, and phenylacetic acid. The Trinitario chocolates were characterized mainly by high DoT factors of acetic acid and esters, with extraordinary high values in Ref3, Ref4, and SB19. The Criollo chocolates showed predominantly high DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1. The similarities indicated an influence of the variety on the flavor compound compositions in dark chocolates. However, although similarities were observed in samples of the same variety, the common features were not exclusive to only one variety.[99] High DoT factors of fruity smelling esters and acetic acid were found in Ref2 (Criollo) and high DoT factors of 2- and 3-methylbutanal, dimethyltrisulfane, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one were observed in a part of the Trinitario and Criollo chocolates. The Nacional chocolate showed clearly higher DoT factors of the three analyzed polyphenols than the Criollo chocolates. Counet et al.[85] analyzed cocoa liquors from different origins and varieties and made a similar observation. While the concentrations of the analyzed pyrazines were predominantly higher in the Forastero liquors, even higher concentrations were found in a Criollo liquor.

Furthermore, we observed variations in the flavor compound compositions of chocolates made from the same variety.[99] These variations were higher in the Trinitario and the Criollo groups than in the Forastero group. Utrilla-Vàzquez et al.[89] reported wide variations in the volatile compositions of Criollo and Trinitario cocoa. They compared three Criollo and two Trinitario cocoas at different processing stages, namely fresh, fermented, and dried. They observed no clear differences in the volatile compositions between the two varieties. Similar to our data,

Quin et al.[83] reported a higher diversity of volatile compositions in Trinitario samples than in Criollo samples. In addition, the Forastero group showed the least variability in the volatile composition in their study.[83]

As known from the literature, the flavor compound compositions of dark chocolates are influenced by many other factors beyond the variety. The growing conditions, fermentation parameters like fermentation time, and other cocoa processing steps such as roasting time and temperature have a strong influence on the flavor and flavor compound profile.[22,24,30,33,80,103] Fermentation time has been associated with the concentration of procyanidins and pyrazines in cocoa liquors.[85] The roasting step is important for the formation of compounds associated with cocoa-like flavor notes such as Strecker aldehydes, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyltrisulfane.[46,47] Conditions during roasting[36] and conching[37] further influence the flavor compound composition. The impact of processing conditions on the flavor-active compounds must be considered for fully processed chocolates and might partially explain the variations in the Trinitario and the Criollo chocolates. Other studies concluded that the process has a greater influence on the volatile composition than the cocoa bean variety.[88,89] However, processing conditions are usually adapted to the cocoa bean quality to obtain the desired flavor profile. For example, the fermentation times of Forastero beans are typically longer than those of Criollo beans.[24,33] Furthermore, different roasting protocols are recommended depending on the variety.[102] Thus, the observed similarities in the flavor compound profiles within one variety could partly result from similar processing conditions of chocolates made from the same variety.[99]

In conclusion, we observed a high variability in the flavor compound compositions of the analyzed chocolates. The variability in the flavor compound profiles within one variety suggested that the variety is not necessarily leading to a specific flavor profile. Common features were observed in chocolates from the same variety, but were not exclusive to this particular variety. We finally concluded that the flavor compound compositions of the single variety dark chocolates were at least partially influenced by the cocoa bean variety.

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8 Appendix

8.1 Publication 1: Impact of water on odor-active compounds in fermented and dried cocoa beans and chocolates made thereof

8.1.1 Bibliographic data

Title:	Impact of water on odor-active compounds in fermented and dried cocoa beans and chocolates made thereof
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8.1.2 Publication reprint

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Impact of Water on Odor-Active Compounds in Fermented and Dried Cocoa Beans and Chocolates Made thereof

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ABSTRACT: The impact of water on odor-active compounds in fermented and dried cocoa beans as well as in chocolate either produced by a novel processing (NPC) or a traditional processing (TPC) technology from the same batch of cocoa beans was investigated in this study. Quantitation of selected key odorants revealed significantly higher concentrations of Strecker aldehydes such as 3-(methylsulfanyl)propanal (66-fold) and phenylacetaldehyde (50-fold) after water treatment of the cocoa beans. The comparison of the two chocolates showed that higher amounts of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde are released with water in the NPC (24-fold to 39-fold), compared to the TPC (7.3-fold−11-fold). In addition to Strecker aldehydes, the concentrations of many further characteristic key odorants of cocoa and chocolate increased after water treatment. Based on the results, a more intense retronasal odor perception of the analyzed compounds is expected due to their release during consumption in contact with saliva.

KEYWORDS: *Theobroma cacao, odorant release, stable isotopically substituted odorants, chocolate, water treatment*

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INTRODUCTION

The main ingredients in a traditional dark chocolate are cocoa liquor, cocoa butter, and sugar. The cocoa liquor is produced by grinding roasted cocoa nibs, and cocoa butter is obtained by pressing cocoa liquor. The mixed ingredients are refined and the mass undergoes a conching step before it is tempered and molded into the final product.[1] Approximately, 600 volatiles have been identified in cocoa and chocolate,[2] but only a minor part of them contributes to the unique aroma. In previous studies, these key odorants were identified and monitored along the cocoa value chain.[3−7] Unfermented cocoa beans contain only small amounts of the key odorants. Some develop during postharvest treatments, especially esters and volatile acids.[3] Roasting leads to formation of Strecker aldehydes, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and pyrazines.[5,7] Some odorants, such as the volatile acids and certain esters, decrease in concentration after roasting.[8] Among all key odorants, acetic acid shows the highest concentration throughout all processing steps and significantly contributes to the chocolate odor.[3−5,9]

Roasting has been considered an essential step in traditional chocolate manufacturing.[1,2] However, using a novel processing technology (NPC), a chocolate with a pleasant sensory profile dominated by flowery and fruity notes as well as low acidity and bitterness can be manufactured without roasting the cocoa beans.[10−12] In brief, fermented and dried cocoa beans are ground with water. In a three-phase decanter, the fat phase is separated from the water phase and the cocoa solids are obtained as a third phase. From the water phase, an aroma fraction is obtained by distillation. The distillation bottoms are spray dried to yield a cocoa extract powder. Thereby, acetic acid is efficiently removed. The different phases are recombined in a defined ratio to form a chocolate mass that is further processed by conching, tempering, and molding. Chetschik et al.[12] compared the key odorant profiles of three chocolates produced by the novel process to the key odorant profiles of six commercially available, traditionally manufactured, single-origin chocolates. The chocolates made with the novel technology showed drastically lower concentrations of acetic acid, 2-methylpropanoic acid, and 2- and 3-methylbutanoic acid than the traditionally processed ones. These acids contribute to the overall cocoa and chocolate odor due to their high odor activity values[5,7,9] and are known to produce off-odors.[13,14] The concentrations of fruity smelling esters like ethyl 2-methylbutanoate and ethyl 3-methylbutanoate were higher in the chocolates made with the novel processing technology than in the traditionally processed chocolates.[12] Interestingly, the authors observed no clear differences in the concentrations of compounds such as Strecker aldehydes and pyrazines that are, according to the literature, formed mainly under thermal treatments like roasting.[2] The authors suggested that these compounds are formed upon water contact from yet unknown, odorless precursors that are already present in the fermented and dried cocoa beans.[12] No attempt to prove this assumption has been reported so far.

However, the release of Strecker aldehydes from roasted cocoa products upon water contact has been observed before.[15,16] Schieberle and Pfnuer[17] reported higher flavor dilution factors and up to 120 times higher concentrations of phenylacetaldehyde in milk chocolate when the volatiles were

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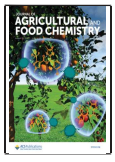

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Table 1. Odorant Concentrations in the Cocoa Nibs before and after Water Treatment with 200% Water for 10 min at 36 °C

odorant	concentration (μg/kg)[a]		factor[b]	odor threshold value	odor activity value	
	before WT	after WT			before WT	after WT
3-(methylsulfanyl)propanal[c]	5.97	394	66	0.52	11	760
phenylacetaldehyde[c]	90.6	4510	50	34	2.7	130
phenylacetic acid[c]	3920	47 200	12	26	150	1800
acetic acid[c]	545 000	4130 000	7.6	350[e]	1600	12 000
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one[c]	12.5	61.8	4.9	0.20[f]	63	310
3-methylbutanal[c]	1650	7300	4.4	15	110	490
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one[c]	357	1560	4.4	27	13	58
3-methylbutanoic acid[c]	8090	34 100	4.2	11	740	3100
2,3,5-trimethylpyrazine[c]	420	1590	3.8	180	2.3	8.8
2-methylbutanoic acid[c]	4520	16 500	3.7	110	40	140
2-phenylethan-1-ol[c]	1430	4840	3.4	490	2.9	9.9
vanillin[c]	35.8	114	3.2	140	<1	<1
2-methoxyphenol[c]	166	406	2.4	1.8[g]	92	230
2-ethyl-3,5-dimethylpyrazine[c]	117	267	2.3	1.7	69	160
4-methylphenol[c]	14.5	31.1	2.1	3.3[g]	4.4	9.4
γ-decalactone[c]	24.3	50.7	2.1	4800	<1	<1
ethyl 3-methylbutanoate[c]	40.3	77.6	1.9	0.98	41	79
3-ethyl-2,5-dimethylpyrazine[c]	63.2	112	1.8	57[f]	1.1	2
3-ethylphenol[c]	12.5	18	1.4	2.2[g]	5.7	8.2
dimethyltrisulfane[c]	45.2	63.6	1.4	0.03	1500	2100
linalool[c]	1010	1420	1.4	3.4	300	420
2-methylbutanal[c]	1770	2250	1.3	34	52	66
2-isobutyl-3-methoxypyrazine[c]	0.356	0.445	1.3	0.04	8.9	11
2,3-diethyl-5-methylpyrazine[c]	1.97	2.46	1.3	7.2	<1	<1
ethyl phenylacetate[c]	384	471	1.2	300	1.3	1.6
2-methyl-3-(methyldisulfanyl)furan[c]	2.64	3.18	1.2	0.37	7.1	8.6
ethyl 2-methylbutanoate[c]	35.4	39.6	1.1	0.37	96	110
ethyl (2*E*)-3-phenylprop-2-enoate[c]	176	189	1.1	7100[h]	<1	<1
ethyl 3-phenylpropanoate[c]	46.7	44.1	0.94	240	<1	<1
3-methylbutyl acetate[d]	1310	1200	0.92	76	17	16
δ-decenolactone[c]	93.5	80.7	0.86	590[i]	<1	<1
octanal[c]	30.7	23.6	0.77	140	<1	<1
2-phenylethyl acetate[c]	1280	849	0.66	14 000	<1	<1

[a]Concentration values are means of triplicates with a relative standard deviation <15%. [b]Factor by which the concentration increases after water treatment. [c]Significant difference. [d]No significant difference in concentration for this compound before and after water treatment ($\alpha = 0.05$) observable. [e]Threshold according to ref 28. [f]Threshold according to ref 29. [g]Threshold according to ref 30 (in deodorized cocoa butter). [h]Threshold according to ref 9. [i]Threshold according to ref 4.

isolated by simultaneous steam distillation extraction, compared to extraction with a low boiling solvent followed by a gentle high vacuum distillation without heat application and water contact. Buhr et al.[15] found ∼10-fold higher concentrations of 2- and 3-methylbutanal and 47-fold higher concentrations of phenylacetaldehyde after water treatment of roasted cocoa beans.[15] They suggested the release from stable intermediates of the Strecker reaction. It was assumed that the additional amounts of Strecker aldehydes generated upon contact with saliva during consumption vitally impact the retronasal odor perception.[15] Granvogl et al.[16] observed increases in the same range for 2- and 3-methylbutanal when adding human saliva to dark chocolate. They showed in model experiments that oxazolines are potent Strecker aldehyde precursors and identified 2-isobutyl-5-methyl-3-oxazoline as a precursor of 3-methylbutanal. However, the concentration of this compound in the chocolate was very low and could only partially explain the observed increase of 3-methylbutanal. There is no study available that analyzes the behavior of Strecker aldehydes upon water treatment in unroasted cocoa products.

In this study, we analyzed the concentrations of Strecker aldehydes and other important cocoa key odorants in fermented and dried cocoa beans before and after water treatment to verify a release by water as hypothesized in a previous publication.[12] Furthermore, the impact of water on the concentrations of major chocolate odorants was investigated with a focus on the differences between a chocolate made by the traditional process (TPC) and a chocolate made by the novel processing technology (NPC).

MATERIALS AND METHODS

Cocoa Nibs and Chocolates. The fermented and dried cocoa nibs (Trinitario variety) originated from Costa Rica. The NPC was a commercially available chocolate with 78% cocoa (Oro de Cacao AG, Zürich, Switzerland). An analogue TPC with 78% cocoa was made from the same batch of cocoa nibs at a laboratory scale (TPC). The internal protocol was developed at the ZHAW and mimicked the traditional chocolate manufacturing process. The cocoa nibs were frozen in liquid nitrogen and milled to a fine powder (<2 mm). The cocoa powder was roasted in a thin layer in between aluminum foils for 7 min at 125 °C in a kitchen hot air oven to enable a homogeneous roasting of the cocoa material. The roasted cocoa

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powder was mixed with sugar and the mixture was processed with a three-roll refiner (Bühler AG, Uzwil, Switzerland). Deodorized cocoa butter (Barry Callebaut, Dübendorf, Switzerland) was added to the cocoa liquor and the mass was tempered before being molded to bars. The chocolate was hardened in a refrigerator (8 °C). The TPC finally consisted of 70.2% cocoa mass, 7.8% deodorized cocoa butter, and 22% sugar. NPC and TPC were stored at 12 °C.

Odorants. The stable isotopically substituted odorants 2-($^2H_3$)-methylbutanal, 3-($^2H_3$)methyl(3,4,4,4$^2H_4$)butanal, ethyl 2-($^2H_3$)-methylbutanoate, ethyl 3-($^2H_3$)methyl(2,2,3,4,4,4-$^2H_6$)butanoate, 3-methylbutyl ($^{13}C_2$)acetate, (7,7,8,8-$^2H_4$)octanal, ($^2H_6$)-dimethyltrisulfane, 2-($^3H_2$)methyl-3,5-dimethylpyrazine, 3-(($^2H_3$)-methylsulfanyl)propanal, 2-(1,1-$^2H_2$)ethyl-3(1,1-$^2H_2$)ethyl-5-($^2H_3$)-methylpyrazine, 3-($^2H_5$)ethyl-2,5-dimethylpyrazine, 2-($^2H_3$)methoxy-3-(2-methylpropyl)pyrazine, 3-($^2H_3$)methyl-7-methyl-(4,4-$^2H_2$)octa-1,6-dien-3-ol, phenyl($^{13}C_2$)acetaldehyde, 2-methyl-3-(($^2H_3$)-methyldisulfanyl)furan, 3-($^2H_3$)methyl(2,2,3,4,4,4-$^2H_6$)butanoic acid, ethyl ($^2H_5$)phenylacetate, 2-($^2H_5$)phenylethyl acetate, 2-($^2H_3$)-methoxyphenol, ($^2H_5$)ethyl 3-phenylpropanoate, 2-($^2H_5$)-phenylethan-1-ol, 4-hydroxy-2-methyl-5-(^{13}C)methyl(5-^{13}C)furan-3(2*H*)-one, 4-methyl(2,6-$^2H_2$)phenol, ethyl (2*E*)-3-($^2H_5$)phenyl-(2,3-$^2H_2$)prop-2-enoate, 5-(1,1-$^2H_2$)hexyl-(3,3,4,4,5-$^2H_5$)oxolan-2-one, 4-ethyl(2,6-$^2H_2$)phenol, 3-hydroxy-4-methyl-5-(^{13}C)methyl-(5-^{13}C)furan-2(5*H*)-one, 6-(4,5-$^2H_2$)pentyloxan-2-one, phenyl($^{13}C_2$)-acetic acid, and 4-hydroxy-3-($^2H_3$)methoxybenzaldehyde were purchased from AromaLAB (Planegg, Germany). ($^{13}C_2$)Acetic acid was purchased from Merck KGaA (Darmstadt, Germany).

The reference odorants 2-methylbutanal, 3-methylbutanal, ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, octanal, dimethyltrisulfane, 2,3,5-trimethylpyrazine, 2-ethyl-3,(5 or 6)-dimethylpyrazine, acetic acid, 3-(methylsulfanyl)propanal, 2,3-diethyl-5-methylpyrazine, 3-isobutyl-2-methoxypyrazine, linalool, phenylacetaldehyde, 2-methyl-3-(methyldisulfanyl)furan, 2-methylbutanoic acid, 3-methylbutanoic acid, ethyl phenylacetate, 2-phenylethyl acetate, 2-methoxyphenol, ethyl 3-phenylpropanoate, 2-phenylethan-1-ol, 4-methylphenol, ethyl (2*E*)-3-phenylprop-2-enoate, γ-decalactone, 3-ethylphenol, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, δ-decenolactone, phenylacetic acid, vanillin, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one were purchased from Merck KGaA.

Other Chemicals. Dichloromethane (GC Ultra grade, purity ≥99.9%), anhydrous sodium sulfate, sodium bicarbonate, and hydrochloric acid were purchased from Carl Roth (Roth AG, Arlesheim, Switzerland). Diethyl ether (Merck KGaA) was freshly distilled before use.

Sample Work-Up. Fermented and dried cocoa nibs (0.5−50 g) were frozen with liquid nitrogen and milled with a laboratory mill (IKA-Werke GmbH & Co. KG, Staufen, Germany). The chocolate (0.5−50 g) was broken into pieces by hand. Extraction was performed by stirring the cocoa nib powder and the chocolate with an adequate amount of solvent (50−250 mL of dichloromethane or diethyl ether). Stable isotopically substituted odorants were used as internal standards and added in the beginning of extraction in an amount that was expected for the target odorant in the sample (0.015−194 μg). The mixture was stirred at room temperature for at least 12 h. Preliminary experiments had verified that the maximum extraction yield was reached after 12 h (Figure S1). The extract was filtered through a folded paper filter and subjected to SAFE distillation.[18] The thawed SAFE distillate was dried over anhydrous sodium sulfate and concentrated to a volume of 300 μL using a Vigreux column and, in the last step, a gentle stream of nitrogen. The 50 g samples were additionally fractionated after SAFE to separate the acidic volatiles from neutral and basic compounds. Fractionation enabled quantitation of minor compounds that could not be chromatographically separated from major volatile acids. The SAFE distillate was first shaken with an aqueous sodium bicarbonate solution (0.5 mol/L) in three portions (150, 50, and 50 mL). The dichloromethane phase, containing the neutral and basic constituents, was dried over anhydrous sodium sulfate and concentrated to 300 μL. The combined aqueous phases were adjusted to a pH below 3 with hydrochloric acid (4 mol/L). The acidic compounds were re-extracted with dichloromethane in four portions (100, 100, 50, and 50 mL). The combined organic phases were dried over anhydrous sodium sulfate and concentrated to 300 μL.

Water Treatment. The cocoa nibs or chocolates (1−10 g) were milled with an analysis mill (IKA-Werke GmbH & Co. KG). The powder was mixed with a double amount of water (2−20 mL). The slurry was stirred magnetically in an Erlenmeyer flask for 10 min in a tempered water bath (36 °C). On the basis of preliminary investigations (Figure S2), these parameters were considered adequate to achieve an exhaustive release of Strecker aldehydes. Anhydrous sodium sulfate (20−150 g) was added and volatiles were subsequently extracted following the protocol described in the abovementioned section. Fractionation was only applied to the 2 g samples among the water-treated samples.

Quantitation of Odorants by Gas Chromatography-Mass Spectrometry (GC-MS). Quantitation was done either with a GC-MS system (I) or with a GC-GC-MS system (II) depending on the target compound. Details are provided in the Supporting Information (Table S1). The GC-MS system consisted of a TRACE GC Ultra (Thermo Fisher Scientific, Reinach, Switzerland) coupled to a TSQ Quantum mass spectrometer (Thermo Fisher Scientific) operating in an EI mode. Volatiles were separated on a DB-FFAP column (30 m length, 0.25 mm inner diameter, 0.25 μm film thickness; Agilent Technologies, Basel, Switzerland) using a constant helium carrier flow of 2.5 mL/min. An autosampler injected 1 μL directly cold on-column. The GC oven was tempered at 40 °C for 6 min, then increasing to 7 °C/min until 240 °C was reached. The MS transfer line was heated to 280 °C, the ion source temperature was 200 °C, and the electron ionization energy was 70 eV. The GC-GC-MS system was the same as previously described.[12] Deviating parameters were an injection volume of 1 μL, a temperature of 240 °C for the transfer line between the two GCs, and the temperature and gas flow programs of both GCs. The oven temperature programs started with 40 °C for both columns except for the measurements of 2-methylbutanal and 3-methylbutanal with 30 °C as initial temperature for the second oven. The first oven started to heat up after 4 min, further increasing to 8 °C/min until 240 °C was reached. The second oven started heating 4 min after the end time of the heart cut, increasing to 8 °C/min up to 200 °C and then increasing to 50 °C/min until 270 °C was reached. The end temperatures were held until the end of the run. The cut time was variable and depended on the retention times of the target compounds on the first column. A cold trap (Brechbühler AG, Schlieren, Switzerland) in the second oven was operated at −140 °C until 0.1 min after the cut and was then rapidly heated up to 220 °C. The front inlet pressure was 180 kPa and the middle inlet pressure was 150 kPa. The middle inlet fed helium directly to the MCSS. The pressures were increased to 250 kPa for the middle inlet and 280 kPa for the front inlet 0.1 min after the heart cut. The MS in both systems was operated in the selected ion monitoring mode with individual quantifier ions for the analyte and the standard for each target compound. The odorant concentration in the sample was calculated by means of the peak areas of the analyte and the standard, the amount of the added standard, and the sample weight by employing calibration line equations. Mixtures of the analyte and the standard in at least five different ratios (5:1, 2:1, 1:1, 1:2, and 1:5) were measured with the above-described methods, and the peak area ratios were calculated to obtain a calibration line equation by linear regression. For each target compound, the quantifier ions, the calibration line equation, and the quantitation system used are detailed in the Supporting Information (Table S1).

Odor Threshold Values. Unless otherwise stated, odor threshold values were determined orthonasally in low odor sunflower oil by the trained panel of the Leibniz Institute for Food Systems Biology at the Technical University Munich according to the American Society for Testing and Materials (ASTM) standard practice for determination of odor and taste thresholds by a forced-choice ascending concentration series method of limits.[19] The number of assessors varied between 15 and 20. The tests were carried out in separate booths of a special room exclusively dedicated to sensory evaluations at 22 ± 2 °C room temperature.

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Statistics. A *t*-test was applied to evaluate if there was a significant difference in the odorant concentration before and after water treatment. An *F*-test evaluated the significance of differences in concentrations after different extraction times. Both tests were carried out with a level of significance of $\alpha = 0.05$, using Python 3.8.3.

RESULTS AND DISCUSSION

Impact of Water on Odor-Active Compounds in Fermented and Dried Cocoa Beans. A total of 33 odor-active compounds that are known to contribute to cocoa and chocolate odor[3−6,9,12] were quantitated in the cocoa nibs before and after water treatment (Table 1). The water treatment had by far the highest impact on the concentrations of the two Strecker aldehydes 3-(methylsulfanyl)propanal (66-fold increase) and phenylacetaldehyde (50-fold increase). The increase of phenylacetaldehyde was similar to the one found by Buhr et al.[15] in roasted cocoa beans (47-fold increase), but the absolute concentrations were lower in our study. The high increase in 3-(methylsulfanyl)propanal and phenylacetaldehyde concentrations indicates a release from precursors as already described for roasted cocoa products in the literature.[15,16] These precursors are assumed to be formed as part of the Strecker reaction during thermal processing.[15,16] It is yet unclear if such compounds are already present in fermented and dried cocoa before roasting. Amadori products might also play a role in the formation of Strecker aldehydes upon water treatment. The concentrations of Amadori compounds were shown to increase drastically during fermentation and drying.[20] Their importance in the thermal formation of Strecker aldehydes has been shown,[20,21] and Granvogl et al.[16] postulated that oxazolines can be directly formed from Amadori products by oxidative decarboxylation. The Amadori compounds could additionally be of major importance in the nonthermal formation of Strecker aldehydes upon water treatment in unroasted cocoa.

The fact that Strecker aldehydes are not only formed as a result of the high temperatures applied during roasting[5] but can also be released from unroasted cocoa by the addition of water explains the similar concentrations of Strecker aldehydes in the chocolates made with the novel processing technology and the traditionally manufactured chocolates reported in the previous study.[12] In addition to Strecker aldehydes, the water treatment resulted in higher concentrations of further important cocoa odorants. The impact on the carboxylic acids was especially high with an increase up to 12-fold for phenylacetic acid. Thus, a specific effect on the group of carboxylic acids can be assumed. Carboxylic acids might be partly present in the form of salts in cocoa and thus would not be extracted completely with a nonpolar solvent like dichloromethane alone. The addition of water could lead to the protonation of the carboxylates and consequently lead to a better extraction yield.

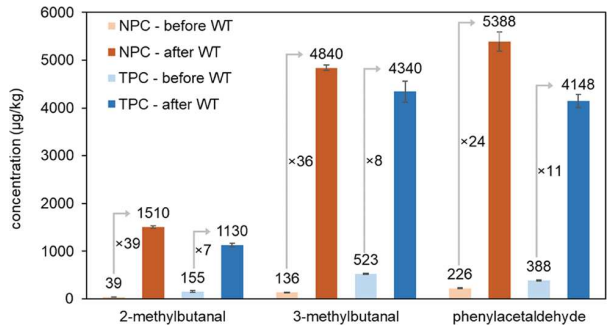

Figure 1. Concentrations of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde before and after water treatment (WT) with 200% water for 10 min at 36 °C in NPC and TPC. Concentrations were analyzed in triplicate; standard deviations were <15%.

The increase in concentrations of odorants with other chemical structures indicates further mechanisms except for the release from specific precursors. For example, odorants might be bound to cocoa matrix components by physical interactions. It has previously been shown that odorants can interact with different food macromolecules.[22] The addition of water could break the interactions between odorant and macromolecule. The resulting odorant release would lead to an improved extraction yield by a subsequent solvent addition. Odorants could bind to polysaccharides, fat, and proteins, but also to cocoa polyphenols. Interactions of ethyl benzoate and polyphenols were observed in a cocoa bean tannin extract.[23] The binding affinity was much stronger than for benzaldehyde and 2-phenylethan-1-ol with the polyphenols. These kinds of interactions seem, therefore, to depend highly on the structure of the odorant.

An impact of water on odor-active compounds was observed in other foods before. Concentrations of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and 2-phenylethan-1-ol increased when hot water was added to black tea leaves.[24] Guth and Grosch[25] observed increased concentrations of odorants like vanillin in oat extrudates after soaking in water. They suggested that interactions with matrix components, specifically starch inclusion complexes, were responsible. More recently, Rögner et al.[26] studied the impact of water addition on malt powder odorants. Concentrations increased 33-fold for phenylacetaldehyde, 14-fold for phenylacetic acid, and 9-fold for vanillin. They suggested a general release mechanism for all volatiles in addition to the release of Strecker aldehydes from specific precursors as discussed before. The occurrence of general release mechanisms that affect odorants of various structures during the contact of food with water is also supported by our results. However, the exact types of interactions that are cleaved upon contact with water and are responsible for the observed concentration increases are not elucidated yet.

Impact of Water on Odor-Active Compounds in Two Differently Processed Chocolates. It was shown that major amounts of Strecker aldehydes are released in traditionally processed chocolates upon contact with water or saliva.[15,16] The in-mouth generation of Strecker aldehydes during chocolate consumption is considered to play a vital role in the retronasal odor perception. In contrast to traditional chocolate manufacturing, the fermented and dried cocoa beans come in contact with water already during processing when the novel technology is applied.[10−12] Given our previous results, it could be expected that this leads to an exhaustive release of odorants from hydrolabile precursors. A significantly lower release of odorants upon contact with saliva during consumption of the chocolate could be the consequence leading to a less intense retronasal odor perception and thus to a reduced eating pleasure.

To challenge this hypothesis, the impact of water treatment on odorant concentrations in an industrially manufactured chocolate using the novel technology (NPC) and a traditionally manufactured model chocolate (TPC) produced from the same batch of cocoa nibs was investigated. Selected chocolate

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Table 2. Selected Odorant Concentrations in the Two Differently Processed Chocolates before and after Water Treatment with 200% Water for 10 min at 36 °C[b]

odorant	concentration in the NPC (μg/kg)			concentration in the TPC (μg/kg)		
	before WT	after WT	factor[a]	before WT	after WT	factor[a]
2-methylbutanal	39	1510	39	155	1130	7.3
3-methylbutanal	136	4840	36	523	4340	8.3
3-(methylsulfanyl)propanal	4.37	140	32	5.76	307	53
phenylacetaldehyde	226	5390	24	388	4150	11
acetic acid	98 900	1530 000	15	244 000	2450 000	10
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one	2.92	24.4	8.4	15.2	49.3	3.2
3-methylbutanoic acid	831	5080	6.1	8380	16 100	1.9
2-methylbutanoic acid	421	1860	4.4	4270	7020	1.6
phenylacetic acid	3360	14 800	4.4	10 000	26 000	2.6
4-methylphenol	12.3	34.2	2.8	18.2	31.7	1.7
4-hydroxy-2,5-dimethylfuran-3(2*H*)-one	193	520	2.7	1210	1680	1.4
2-ethyl-3,5-dimethylpyrazine	41.4	96.5	2.3	139	203	1.5
2-phenylethan-1-ol	815	1620	2	2010	2590	1.3
2-methoxyphenol	88.2	139	1.6	172	238	1.4
2,3,5-trimethylpyrazine	250	332	1.3	1310	885	0.67

[a]Concentration values are means of triplicates with a relative standard deviation <15%. [b]Factor by which the concentration increased after the water treatment. The change after the water treatment was statistically significant (α = 0.05) for all odorants in NPC and TPC.

odorants were quantitated before and after water treatment. The selection included Strecker aldehydes and further odorants, which had shown a significant concentration increase upon water treatment and high odor activity values in the fermented and dried cocoa nibs.

Interestingly, the NPC behaved not as expected but released even higher amounts of Strecker aldehydes upon water treatment than the TPC (Figure 1). The highest increase was observed for the compound 2-methylbutanal (39-fold). Consequently, the NPC contained higher concentrations of Strecker aldehyde precursors even though the cocoa beans had already been in contact with water during processing. This suggested a more intense retronasal odor perception compared to the TPC regarding malty and honey-like sensations caused by the in-mouth generation of these Strecker aldehydes during consumption. Not only Strecker aldehydes increased upon water contact of the chocolates, but also other odorants (Table 2). It can be hypothesized that the odorants released by water contact in the early stages of the novel process are, later on in the process, again bound by the formation of hydrolabile complexes. In this way, they could be released again by water treatment of the final chocolate.

The Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde show similar concentrations in the NPC and the TPC after water treatment. The concentrations of other odorants are mostly higher in the TPC. Higher concentrations of compounds such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and 2,3,5-trimethylpyrazine in the TPC are in line with their formation during roasting. Lower concentrations of carboxylic acids in the NPC are the result of the efficient removal in the novel processing technology and were reported before.[12]

Finally, the results of this study demonstrate the great impact of water on the release of Strecker aldehydes and other key odorants in cocoa and chocolate. This is in accordance with the previously discussed studies on roasted cocoa, chocolate, and other foods.[15,16,24−26] In the majority of previous studies, the isolation of volatiles from cocoa products did not include a water treatment prior to the sample work-up.[4−6,9,12] Volatiles were extracted directly with a low boiling, organic solvent, and an additional odorant formation upon water contact was not investigated. Already, Buhr et al.[15] concluded that a sample work-up without water could underestimate the importance of certain compounds like Strecker aldehydes because it does not consider their in-mouth generation during consumption. However, it is important to differentiate between the orthonasal and the retronasal odor perception.[27] The release of additional amounts of odorants with water or saliva is of major importance for the retronasal odor perception. The orthonasal odor perception, however, is only impacted by the odorant concentrations that migrate into the gas phase from the matrix without any added water.

In conclusion, a water-free sample work-up is adequate to characterize the orthonasal odor profile, whereas water treatment prior to the sample work-up is essential to represent the retronasal odor profile.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.1c02287.

(Figure S1) Extraction yields of selected odorants in the relative percentage of the maximum concentration after different extraction periods (1, 4, 12, and 24 h), (Figure S2) the concentration of phenylacetaldehyde after different periods of water treatment (0−15 min), (Figure S3) the concentration of phenylacetaldehyde after addition of different amounts of water (0−200% relative to the sample weight), and (Table S1) stable isotopically substituted odorants and parameters used in the quantitation of odor-active compounds (PDF)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

GC-MS, gas chromatography-mass spectrometry; MS, mass spectrometer; NPC, chocolate made with the novel processing technology; SAFE, solvent-assisted flavor evaporation; TPC, chocolate made with the traditional processing technology; WT, water treatment

■ NOMENCLATURE

vanillin, 4-hydroxy-3-methoxybenzaldehyde; γ-decalactone, 5-hexyloxolan-2-one; linalool, 3,7-dimethylocta-1,6-dien-3-ol; 2-isobutyl-3-methoxypyrazine, 2-methoxy-3-(2-methylpropyl)-pyrazine; δ-decenolactone, 6-pentyl-5,6-dihydro-2*H*-pyran-2-one

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8.1.3 Summary and individual contributions

In traditional chocolate production, roasting is considered an essential step for flavor formation. In a novel chocolate making technology, fermented and dried cocoa beans are not roasted, but ground with water before being further processed. In a previous study, chocolates made by the novel process (NPCs) and traditionally processed chocolates (TPCs) showed comparable concentrations of compounds known from the literature to be mainly formed during roasting, such as Strecker aldehydes. The authors hypothesized that these compounds were released upon water contact from yet unknown precursors in the cocoa beans in the novel process. Our study aimed to test this assumption by investigating the impact of water on the concentrations of Strecker aldehydes and other cocoa key odorants in fermented and dried cocoa beans. Furthermore, it was not clear whether similar amounts of odor-active compounds are released by water in NPCs as in TPCs, as the cocoa beans were already in contact with water during processing. Therefore, the influence of water on selected odorants in an NPC and a TPC, both made from the same batch of cocoa beans, was additionally investigated.

Selected odorants were quantitated in fermented and dried cocoa beans before and after water treatment by GC-MS using isotopically substituted odorants as internal standards. The highest increases in concentration by water treatment were observed for the two Strecker aldehydes 3-(methylsulfanyl)propanal (66-fold) and phenylacetaldehyde (50-fold). The high increase factors indicated the release from specific precursors in the fermented and dried cocoa beans upon water contact. Water treatment also resulted in higher concentrations of most other quantitated odorants with high increase factors for carboxylic acids such as phenylacetic acid (12-fold) and acetic acid (7.6-fold). The effect of water on a wide range of odorants suggested other mechanisms in addition to the release from specific precursors. Cocoa odorants may be physically bound to cocoa matrix components such as polysaccharides, fat, and proteins. We assumed that these interactions were interfered by water, leading to a higher extraction yield. Consequently, the release of odorants in fermented and dried cocoa beans by water explained the similar concentrations of Strecker aldehydes in NPCs and TPCs. Interestingly, the quantitation of odor-active compounds in the NPC and the TPC revealed higher increases in the concentrations of nearly all quantitated odorants upon water treatment in the NPC than in the TPC. The results suggested that odorants were released during the water treatment in the novel processing and were bound at a later stage in such a way that they were released again in the chocolate by water contact. Our results further demonstrated that water treatment before sample work-up is essential for representing the retronasal odor profile, while a water-free sample work-up should be chosen for characterizing the orthonasal odor profile.

Lisa Ullrich designed and performed the experiments including production of the TPC, sample work-up, quantitation, and calculation of OAVs. Lisa Ullrich further summarized, illustrated, and interpreted the data, and prepared the manuscript. Silva Neiens developed the water treatment method applied in this study. Tilo Hühn participated in the discussion and interpretation of the results and revised the manuscript. Martin Steinhaus designed the experiments, interpreted the results, and revised the manuscript. Irene Chetschik designed and supervised the experiments, and interpreted the results. In addition, she assisted in preparing the manuscript, revised it, and submitted it.

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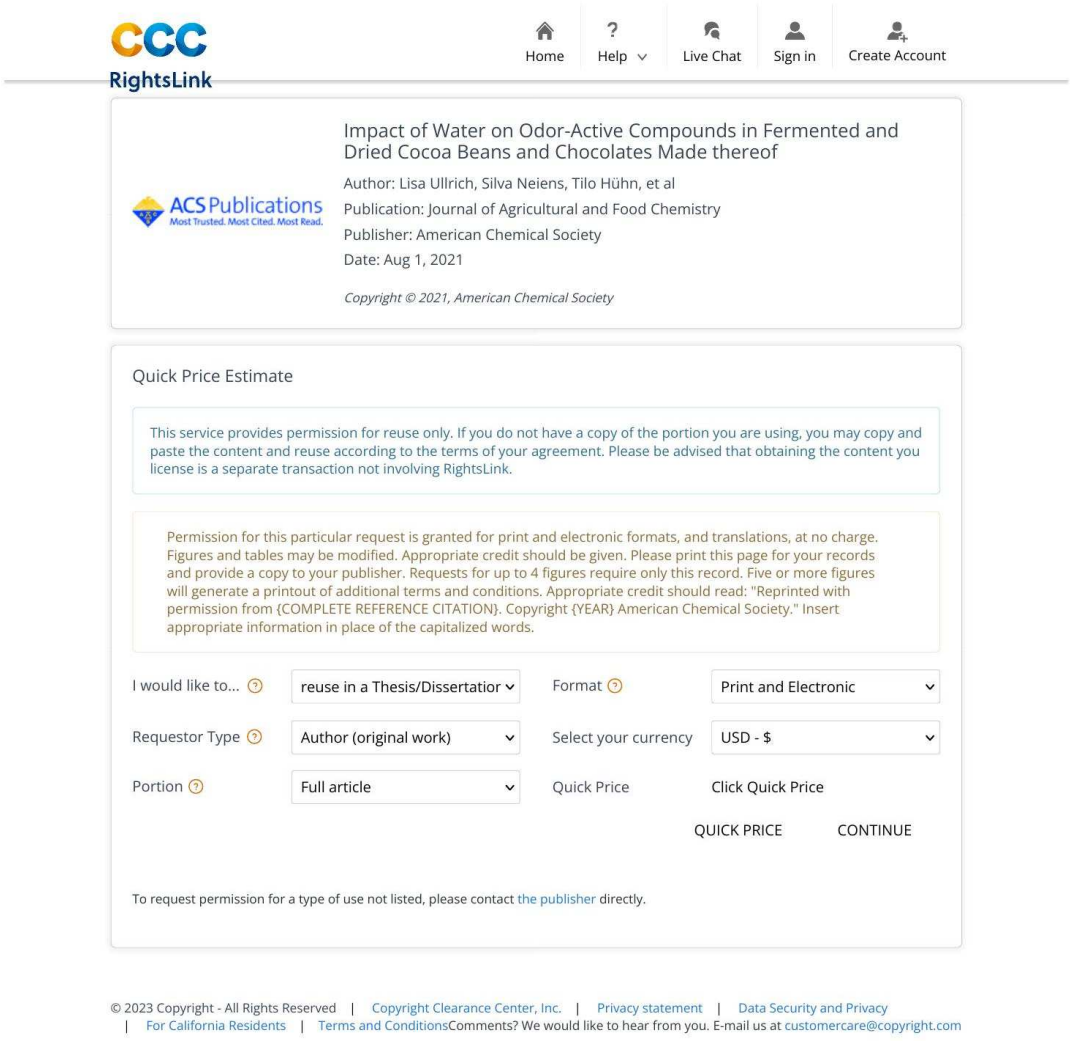

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8.2 Publication 2: Decoding the fine flavor properties of dark chocolates

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8.2.2 Publication reprint

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Decoding the Fine Flavor Properties of Dark Chocolates

Lisa Ullrich, Bettina Casty, Amandine André, Tilo Hühn, Martin Steinhaus,* and Irene Chetschik*

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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 flavors were 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*

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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 from 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 that are produced on a 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 increasing. 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 that 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 a 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 result, they found certain odorants associated with four different groups. Liu et al.[23] analyzed two chocolates and a cocoa liquor with a 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

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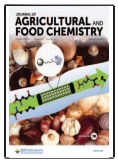

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associated with a fruity odor. Rottiers et al.[24] analyzed liquors of four EET cultivars and one CCN51 sample and performed both a sensory characterization and a semiquantitation of volatiles with headspace-solid-phase microextraction-gas chromatography-mass 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, nonvolatile 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 the 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).

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

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, methylbutanoate, 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-($^2H_3$)methylbutanal, 3-($^2H_3$)methyl(3,4,4,4$^2H_4$)butanal, phenyl($^{13}C_2$)acetaldehyde, 3-($^2H_3$)methyl(2,2,3,4,4,4-$^2H_6$)butanoic acid, phenyl($^{13}C_2$)acetic acid, 3-methylbutyl ($^{13}C_2$)acetate, ethyl 2-($^2H_3$)methylbutanoate, ethyl 3-($^2H_3$)methyl(2,2,3,4,4,4-$^2H_6$)butanoate, ethyl ($^2H_5$)phenylacetate, 2-($^2H_5$)phenylethyl acetate, ethyl (2*E*)-3-($^2H_5$)phenyl(2,3-$^2H_2$)prop-2-enoate, ($^2H_3$)methyl-7-methyl-(4,4-$^2H_2$)octa-1,6-dien-3-ol, 2-($^2H_5$)-phenylethan-1-ol, 2-(1,1-$^2H_2$)ethyl-3(1,1-$^2H_2$)ethyl-5-($^2H_3$)-methylpyrazine, 3-($^2H_5$)ethyl-2,5-dimethylpyrazine, 2-($^3H_3$)methyl-3,5-dimethylpyrazine, 2-($^2H_3$)methoxyphenol, 4-methyl(2,6-$^2H_2$)-phenol, 2-methyl-3-(($^2H_3$)methyldisulfanyl)furan, 5-(1,1-$^2H_2$)hexyl-(3,3,4,4,5-$^2H_5$)oxolan-2-one, 3-hydroxy-4-methyl-5-(^{13}C)methyl-(5-^{13}C)furan-2(5*H*)-one, ($^2H_6$)dimethyltrisulfane, 4-hydroxy-2-methyl-5-(^{13}C)methyl(5-^{13}C)furan-3(2*H*)-one, 4-hydroxy-3-($^2H_3$)-methoxybenzaldehyde, and 5-(4,4,5,5-$^2H_2$)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. Ten grams of chocolate was diluted in 40 mL of *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 the day after. The residue was frozen for at least 2 h 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 Workup for Gas Chromatography-Olfactometry. Twenty-five grams 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 h, the diethyl ether phase of the extract was separated by means of a separating funnel and a centrifuge (10 min, 11 000 rpm, 14 610*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 Workup 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 (20−400 μL)

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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[e]

			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	methylbutanoate[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.

were added in an amount as expected for the target compounds in the sample. The sample was stirred for at least 12 h 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

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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 Workup 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 15 000 rpm (25 150*g*) for 10 min, and the supernatant was used for the 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 workup 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 the carrier gas and a constant flow of 3 mL/min. One microliter 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 samples ref1, ref4, and ref5. The concentrated distillates of the samples were diluted stepwise with diethyl ether at a ratio of 1:4 to obtain dilutions up to 1:4096. The undiluted and diluted samples were analyzed by GC-O 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 using kits obtained from r-biopharm (Darmstadt, Germany) in combination with a Chemwell 2910 Automated EIA and Chemistry Analyzer (Awareness Technology, Palm City).

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 mm × 100 mm, 2.7 μm) column preceded by a guard column (Agilent EC-18, 2.1 mm × 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 the 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 $\alpha = 0.05$. 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 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 acids, 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 those in 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 ref5 (floral, astringent), 2- and 3-methylbutanoic acids 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 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

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Table 3. Concentrations of Selected Odorants and Tastants in the Six Reference Chocolates as Means of Triplicate (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	11 900	9300
phenylacetaldehyde	2390	1600	2840	1830	4760	3030
2-methylbutanoic acid	4730	6970	7090	5920	11 000	6320
phenylacetic acid	18 200	16 500	32 700	20 600	40 600	25 900
3-methylbutanoic acid	11 500	11 600	20 500	11 200	23 700	14 400
acetic acid	3 310 000	2 370 000	2 330 000	1 750 000	1 460 000	1 660 000
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]

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 the 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 the 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 acids, 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 workup, 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*)-

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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					
		fruity, acidic			floral, astringent	cocoa-like, roasty	
	threshold 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 ref 29. [b]Odor threshold value in μg/kg according to ref 36. [c]Odor threshold value in μg/kg according to ref 15. [d]Odor threshold value in μg/kg according to ref 17. [e]Odor threshold value in μg/kg according to ref 39. [f]Odor threshold value in μg/kg according to ref 19. [g]Taste threshold in μmol/kg for sour. [h]Bitter. [i]Astringent perception according to ref 26. [j]Data were taken from a previous publication.[30]

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 of 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, and 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,

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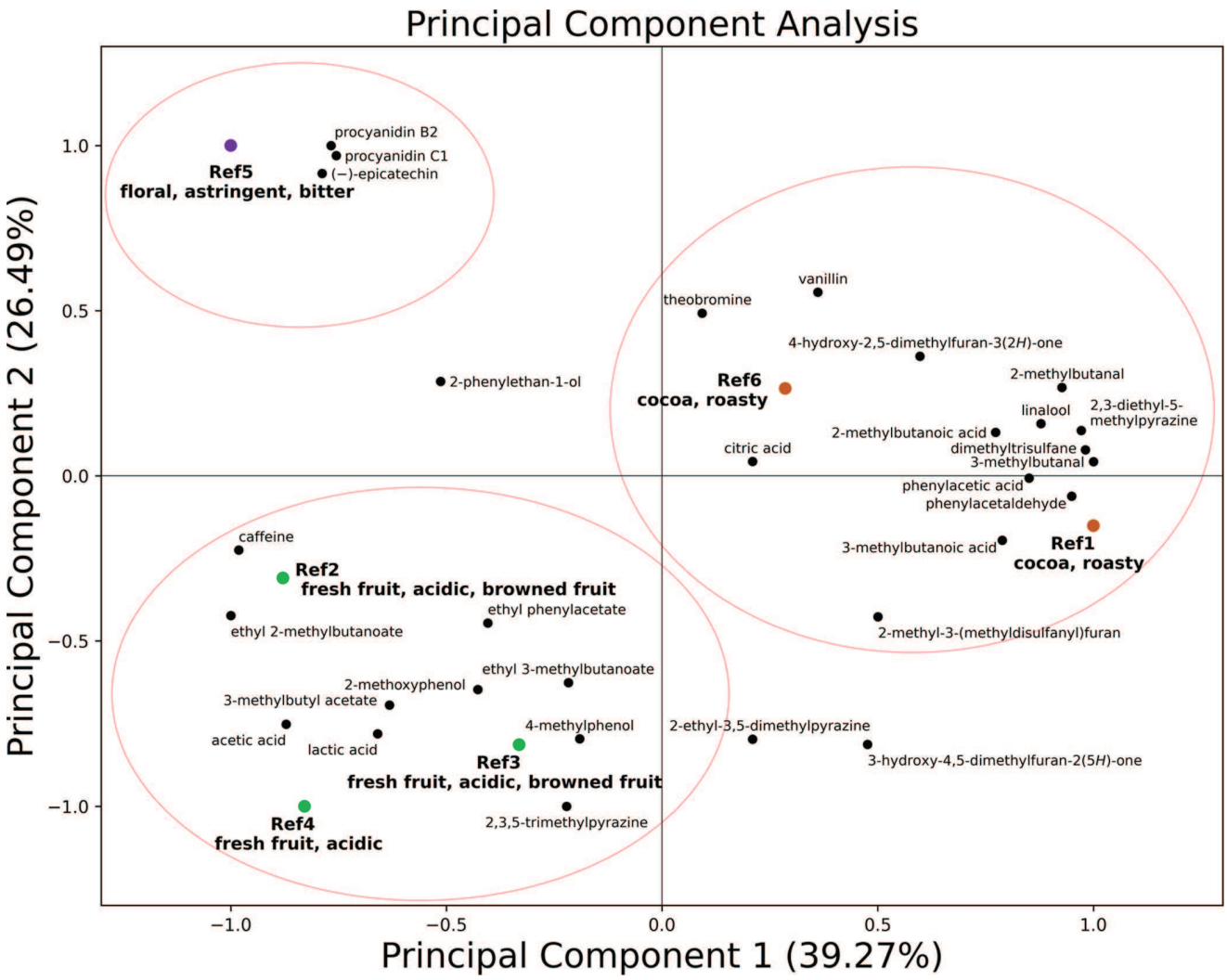

Figure 1. Principal component analysis of flavor-active compounds with DoT factor >1 in the reference chocolates.

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 ref5 (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 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 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 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 by 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.

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The first cluster consisted of the three chocolates ref2, ref3, and 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 that correspond 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 ref4 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 those in 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 those in 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 fruit 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 in 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 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 were 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 acids, 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 that of 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

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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 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 of 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 are 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.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c04166.

Roasting parameters of the reference chocolates (Table S1); stable isotopically substituted odorants and parameters used in the quantitation of odor-active compounds (Table S2); and parameters used in the quantitation of taste-active compounds (Table S3) (PDF)

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Notes

The authors declare no competing financial interest.

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■ 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*]-

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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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8.2.3 Summary and individual contributions

Bean-to-bar chocolates made from fine flavor cocoa of defined variety and origin are appreciated by consumers for their unique flavor profiles. Fine flavors, such as floral, fruity, acidic, and cocoa, are widely described in the literature on a sensory testing level. Initial attempts have been made to link fine flavor attributes of cocoa products to specific compounds, but the molecular background of fine flavor properties was previously not fully understood. The aim of this study was therefore to chemically decode specific fine flavor properties in dark chocolates.

Six dark chocolates with distinct flavor properties were analyzed. They were made from cocoa liquors selected by CoEx as sensory reference samples for specific flavor attributes. Three chocolates showed distinct fruity and acidic flavor notes, two chocolates showed distinct roasty and cocoa-like flavor notes, and one chocolate showed distinct floral and astringent flavor notes. One chocolate of each sensory category was subjected to AEDA. AEDA provided first insights into the molecular background of the different flavor profiles, such as mostly higher FD factors of fruity smelling esters in the chocolate referenced as fruity and acidic. As the main outcome, AEDA showed that the distinct flavor properties must be caused by quantitative differences in already known cocoa key odorants. In the next step, selected odorants and tastants were quantitated in all six chocolates preferably using isotopically substituted compounds as internal standards. DoT factors were calculated to decode the distinct flavor properties on a molecular level. Flavor profiles dominated by fruity and acidic flavor notes seemed to be evoked by acetic acid and a combination of fruity smelling esters such as ethyl 2-methylbutanaote, ethyl 3-methylbutanoate, and 3-methylbutyl acetate. The concentration of acetic acid may thereby have influenced the type of fruity perception regarding fresh fruit and browned fruit notes. Distinct cocoa-like and roasty flavor notes were associated with high DoT factors of the cocoa key odorants 2-methylbutanal, 3-methylbutanal, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyltrisulfane. A floral odor was mainly linked to high DoT factors of 2-phenylethan-1-ol, whereas high DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 indicated an intense astringency.

Lisa Ullrich designed and performed the experiments which mainly included sample work-up, AEDA, quantitation by GC-MS, and calculation of DoT factors. She further evaluated, summarized, interpreted, and illustrated the data, and prepared the manuscript. Bettina Casty designed and performed the experiments together with Lisa Ullrich and also evaluated the data. Amandine André performed the HPLC measurements, evaluated the data, and revised the manuscript. Tilo Hühn participated in the discussion of the results. Martin Steinhaus designed the experiments, interpreted the results, and revised the manuscript. Irene Chetschik designed and supervised the experiments, participated in the GC-O analysis, and interpreted the results. She further assisted in preparing the manuscript, revised it, and submitted it.

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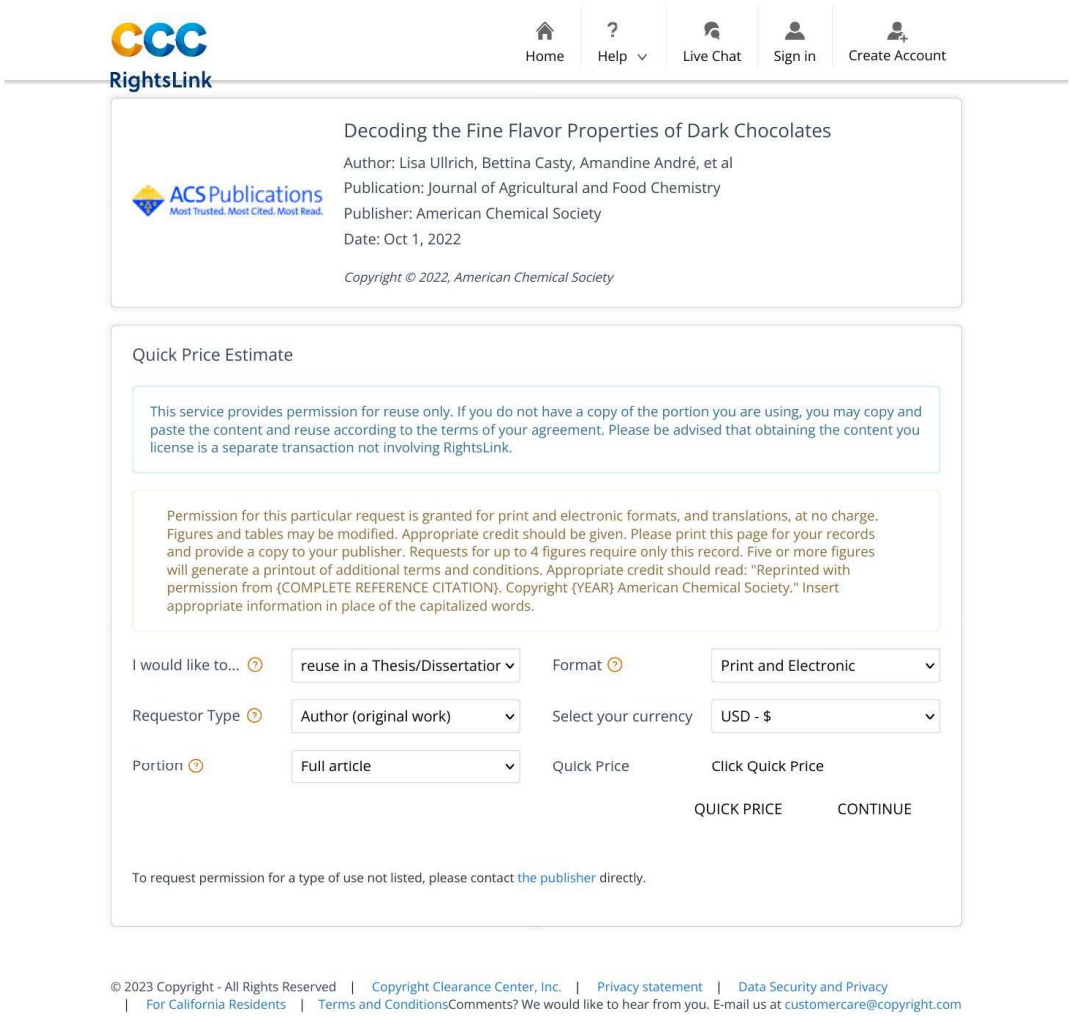

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8.3 Publication 3: Influence of the cocoa bean variety on the flavor compound composition of dark chocolates

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8.3.2 Publication reprint

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Influence of the Cocoa Bean Variety on the Flavor Compound Composition of Dark Chocolates

Lisa Ullrich, Bettina Casty, Amandine André, Tilo Hühn, Irene Chetschik,* and Martin Steinhaus*

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ABSTRACT: The flavor quality is often linked to the cocoa bean variety in the literature although the influence of the variety on the flavor compound composition of chocolate has not been studied comprehensively. To investigate this, dose-over-threshold (DoT) factors of flavor-active compounds in 16 dark chocolates were compared. The three Forastero chocolates were similar and characterized by high DoT factors of 3-methylbutanal, dimethyltrisulfane, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, 2-methylbutanoic acid, 3-methylbutanoic acid, phenylacetic acid, and linalool. However, the wide variations in the flavor compound profiles of the Criollo and Trinitario chocolates suggested that the variety is not the only determinant for the flavor compound composition of dark chocolates. Three Trinitario chocolates and a Criollo chocolate showed especially high DoT factors of fruity smelling esters and acetic acid while others showed similarities to the Forastero chocolates. However, the flavor compound compositions of the single-variety dark chocolates could at least be partly linked to the cocoa bean variety.

KEYWORDS: *Theobroma cacao, cocoa variety, flavor-active compound, dark chocolate, stable isotopically substituted odorant*

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INTRODUCTION

Cocoa is grown around the equator with the main production countries being Côte d'Ivoire, Ghana, and Ecuador.[1] On the cocoa world trade market, cocoa is separated into "fine or flavor" cocoa and "bulk" cocoa.[2] While the demand for fine or flavor cocoa was low in recent decades due to the preference of consumers for filled chocolate products, it has started to grow very rapidly in the last few years.[2] In 2017, less than 5% of the world's production was classified as fine or flavor cocoa.[3] In 2019, about 8% of the worldwide produced cocoa was fine or flavor cocoa[4] and the share increased up to 12% of the total world export of cocoa beans today.[2] The increase in fine or flavor cocoa production is linked to the higher demand of consumers for bean-to-bar products such as small-batch chocolates.[2] Such chocolates are made with cocoa beans of defined variety and origin that are purchased in small batches directly from the farmer. The cocoa beans are processed in small quantities, typically 2−60 kg of chocolate per week,[5] with the aim to create a chocolate with a unique flavor profile.[6]

The flavor is the main criterion for assessing fine or flavor cocoa, and fine flavors include notes like fruity, floral, herbal, woody, nutty, caramel-like, and chocolate base notes.[2] The definition is still controversial as no universally accepted criterion discriminates fine or flavor cocoa from bulk cocoa and objective indicators are still missing.[2] Genetic variety is one criterion among other factors such as morphological characteristics of the plant, chemical composition, degree of fermentation, drying parameters, acidity, contamination with mold or insects, and of course the sensory properties.[2] Traditionally, cocoa is classified as Criollo, Forastero, and Trinitario.[1] However, due to a long history of breeding and hybridization, the genetic background of cocoa is more diverse. Recent studies showed that the genotypes of cocoa can be summarized into about 10 clusters, including Criollo, Nacional, and Amelonado.[7,8] Despite the huge genetic diversity, it is often generalized that bulk cocoa comes from Forastero beans, whereas Trinitario, Criollo, and Nacional are fine or flavor varieties.[2,3] Nevertheless, not all cocoa from the latter varieties is classified as fine or flavor cocoa[2,3] because the genotype alone does not guarantee a high flavor quality, instead both good and poor flavor properties exist within each genetic group.[9] In the literature, the flavor quality is still often linked to the cocoa bean variety and the variety is used as a major criterion in the assessment of cocoa as fine or flavor cocoa. However, the influence of the variety on the flavor compound composition of chocolate has not been studied comprehensively.

Apart from the cocoa variety, also the origin was linked to distinct flavor attributes in the literature.[4,9,10] Previous studies, however, either focused on sensory evaluation[11] or volatile composition.[12−16] Even though in some studies the impact on the odor was partly evaluated by calculating odor activity values, semiquantitative methods using headspace analysis were common and in addition often did not cover all the known key odorants.[17−19] On the other hand, well-designed studies with a focus on sensory-active compounds often analyzed cocoa products with no defined variety.[20−23] However, such products are usually blends from bulk-grade cocoa beans. Chetschik et al.[23] detected differences between

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the flavor profiles of traditionally manufactured chocolates made of cocoa beans of defined origin and chocolates made with cocoa blends. The single-origin chocolates were characterized by very intense fruity notes, whereas the blend chocolates showed more intense roasty, caramel-like, and coconut-like notes. The differences were reflected on the molecular level. Frauendorfer and Schieberle were the first to compare cocoa of different varieties with a focus on sensory-active compounds. They analyzed the changes in key odorants during roasting of both Criollo and Forastero cocoa beans and evaluated the differences between the two varieties as not very pronounced.[24,25] However, the analysis of only two samples does not allow one to draw general conclusions. To the best of our knowledge, no comprehensive study is currently available in the literature that compared chocolates of different cocoa bean varieties with a focus on flavor-active compounds. However, six dark chocolates made from cocoa beans of a defined variety were analyzed in our recent investigation with the aim of decoding the fine flavor attributes of chocolate on the molecular level.[26] To better understand the influence of the cocoa bean variety on the flavor compound composition of dark chocolates, selected odorants and tastants were quantitated in 10 additional commercially available small-batch chocolates of defined variety analogously to our previous investigation.[26]

MATERIALS AND METHODS

Chocolates. Ten small-batch chocolates were purchased from commercial sources. The sample data, including the cocoa bean variety, are listed in Table 1. Sample codes correspond to the chocolates previously analyzed in another investigation.[27] The roasting parameters are provided in the Supporting Information (Table S1).

Table 1. Metadata of Analyzed Small-Batch Chocolates

sample code[a]	bean variety[b]	cocoa bean origin[b]	country of chocolate production	cocoa percentage[c]
SB11	Trinitario	Madagascar	Netherlands	70
SB12	Trinitario	Madagascar	Germany	70
SB7	Criollo	Mexico	Netherlands	70
SB10	Criollo	Mexico	Switzerland	70
SB17	Trinitario	Tanzania	Netherlands	70
SB19	Trinitario	Tanzania	Switzerland	72
SB4	Criollo	Colombia	Netherlands	70
SB6	Criollo	Colombia	Switzerland	80
SB27	Criollo	Peru	Switzerland	75
SB28	Forastero	Ghana	Switzerland	100

[a]Sample codes are not named continuously because the samples were part of a bigger sample set. [b]Cocoa bean variety and cocoa bean origin are taken from producer information. [c]Cocoa percentage as declared on the packaging.

Reference Substances. The 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 cinnamate, 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, and vanillin were purchased from Merck (Darmstadt, Germany). The sources of stable isotopically substituted odorants and reference tastants can be found in our previous publication.[26]

Quantitation of Odorants. The sample work-up and the quantitation parameters for gas chromatography−mass spectrometry (GC−MS) were described previously.[26] In short, chocolate was stirred for ≥12 h with a mixture of water and diethyl ether containing stable isotopically substituted odorants (0.002−3313 μg) as internal standards. The diethyl ether phase was subjected to SAFE, and the distillate was dried over anhydrous sodium sulfate before being concentrated by a Vigreux column and a gentle stream of nitrogen.

Quantitation of Tastants. Lactic acid, citric acid, theobromine, caffeine, (−)-epicatechin, procyanidin B2, procyanidin C1, and cyclo(L-pro-L-val) were quantitated in the chocolate after defatting as described previously.[26]

Statistics. Statistical analysis and data visualization were done with Python 3.8.3.

RESULTS AND DISCUSSION

Data obtained from the quantitation of 27 odorants and 8 tastants in 10 small-batch chocolates (Table S2) were combined with data of 6 chocolates previously analyzed.[26,27] For better comparability between the samples, concentrations were normalized to the cocoa content of the chocolates, which ranged from 70 to 100%. Normalized concentrations in the entire set of 16 samples, including six Criollo, six Trinitario, three Forastero, and one Nacional chocolate according to the traditional classification applied on the trade level[1] are available in the Supporting Information (Table S3). The normalized concentrations were divided by the individual sensory threshold concentrations of the compounds[26] to obtain dose-over-threshold (DoT) factors (Table S4). Ethyl cinnamate, 2-phenylethyl acetate, γ-nonalactone, γ-decalactone, and cyclo(L-pro-L-val) showed DoT factors <1 in all samples, thus were considered of little relevance for the overall flavor profiles and therefore were not included into the following evaluation, although potential additive effects have been discussed.[26] In addition, the only Nacional sample was excluded from the statistical evaluation and will be discussed separately from the Forastero, Trinitario, and Criollo chocolates.

The remaining data, that is the DoT factors of 23 odorants and 7 tastants in the six Criollo, six Trinitario, and three Forastero chocolates, were summarized as a heat map (Figure 1) and were additionally subjected to principal component analysis (PCA) to further visualize differences and similarities in the flavor compound compositions of the samples (Figure 2). The first two principal components of the PCA explained 47.37% of the variance. Both, the heat map and the PCA, indicated similarities in the flavor compound compositions of chocolates made from the same variety, such as the three Forastero chocolates and the three Trinitario chocolates Ref3, Ref4, and SB19. Furthermore, both figures showed a higher variability of the flavor compound compositions in the Trinitario and Criollo chocolates than in the Forastero chocolates.

Flavor-Active Compounds in the Forastero Chocolates. The three chocolates made from Forastero beans clustered together in the heat map (Figure 1) and were linked closely together in the PCA plot (Figure 2), thus indicating the similarities in their flavor compound profiles. The low values of the Forastero chocolates on principal component (PC) 2 were associated with high DoT factors of 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde. Especially 2- and 3-methylbutanal were characteristic for cocoa-like flavor notes in our previous study[26] and both chocolates referenced with

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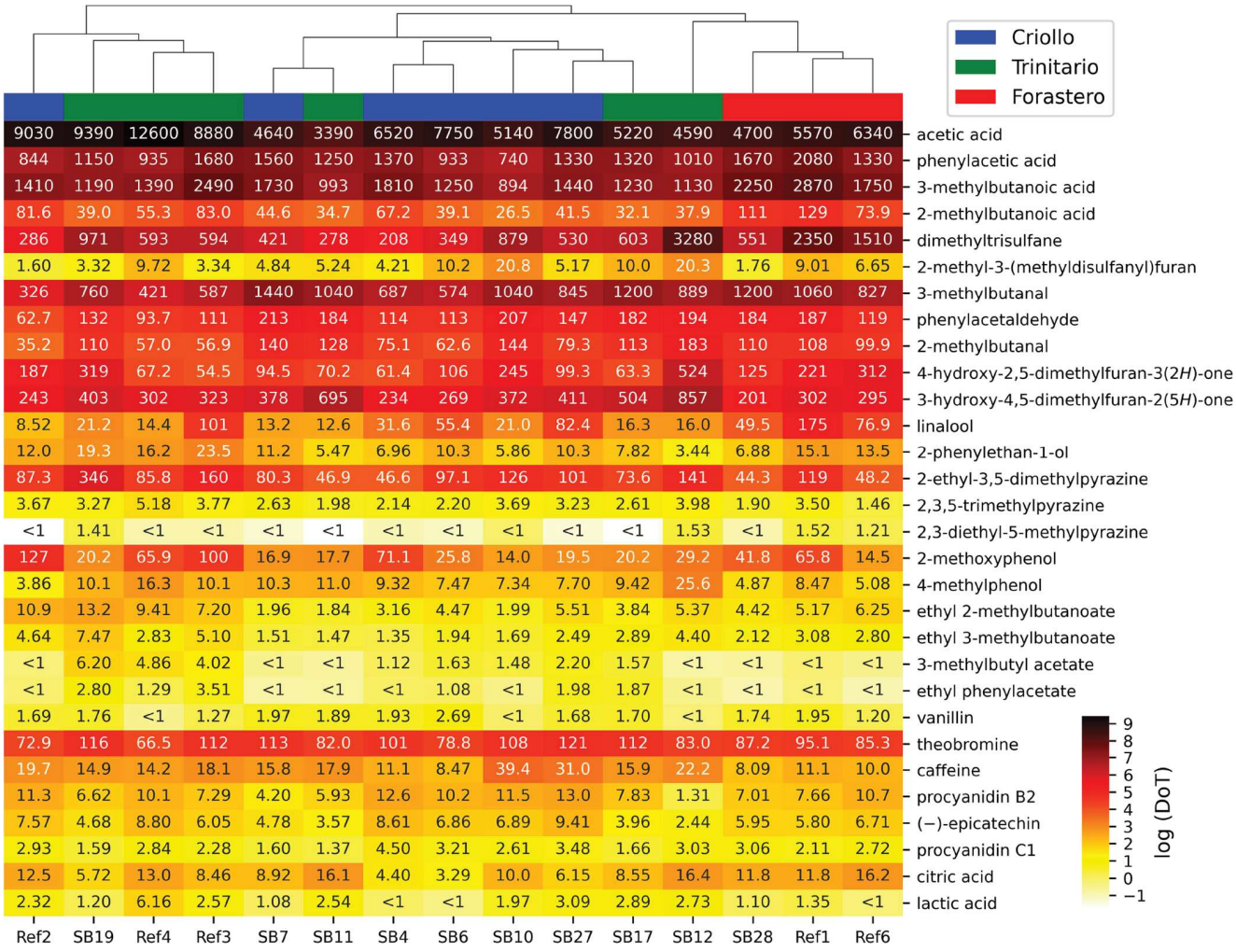

Figure 1. Heat map summarizing the DoT factors of flavor-active compounds in 15 chocolates.

cocoa-like and roasty flavor notes (Ref1, Ref6)[26] were made from Forastero beans. Additionally, the three Forastero chocolates showed mostly high DoT factors of other compounds that were assumed to be responsible for cocoa-like flavor notes such as 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one and dimethyltrisulfane. Our data were in line with previous studies reporting that Forastero beans develop a strong chocolate-like flavor after fermentation and roasting.[3] Although these compounds can be assumed characteristic of Forastero chocolates, many of them were found with even higher DoT factors in chocolates from other varieties (Figure 1). Consequently, these compounds cannot be regarded as exclusive in Forastero chocolates.

The Forastero chocolates, however, showed significantly higher DoT factors of phenylacetic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, and linalool than the Trinitario and the Criollo chocolates when averaging all values from one variety. Linalool is assumed to be a marker for fine flavor cocoa with high concentrations found in Nacional and Criollo cocoa.[28−30] Qin et al.[16] found higher concentrations of linalool in Trinitario and Criollo cocoa than in Forastero cocoa. This is contradictory to the high DoT factors of linalool in the Forastero chocolates of the current study. Tuenter et al.[19] found higher linalool concentrations in liquor and chocolate from a West African blend than in the ones from Nacional cocoa beans. Cambrai et al.[12] determined linalool as a characteristic compound in West African dark chocolates. The three Forastero chocolates analyzed in our study originated all from West Africa. In addition, Frauendorfer and Schieberle[25] found higher linalool concentrations in Forastero cocoa beans than in Criollo cocoa beans. Nevertheless, further investigations are necessary to clarify if high linalool concentrations occur predominantly in Forastero cocoa products.

The DoT factors of the pyrazines were relatively low in all samples. The low impact of pyrazines on cocoa-like flavor notes was also shown previously.[26] In particular, the DoT factors of the pyrazines were not especially high in the Forastero chocolates compared to the ones in the other chocolates, although that might have been expected due to the longer fermentation times and higher roasting temperatures typically applied to Forastero beans.[10,31,32] By contrast, Counet et al.[15] found predominantly higher pyrazine concentrations in two Forastero liquors compared to liquors from other varieties. Tuenter et al.[19] reported higher pyrazine concentrations in liquor and chocolate from a West African blend than in the ones from Nacional cocoa beans. Our findings contradict to these studies but are in line with the ones of Frauendorfer and Schieberle[24,25] who did not observe higher pyrazine concentrations in Forastero cocoa beans than in Criollo cocoa beans.

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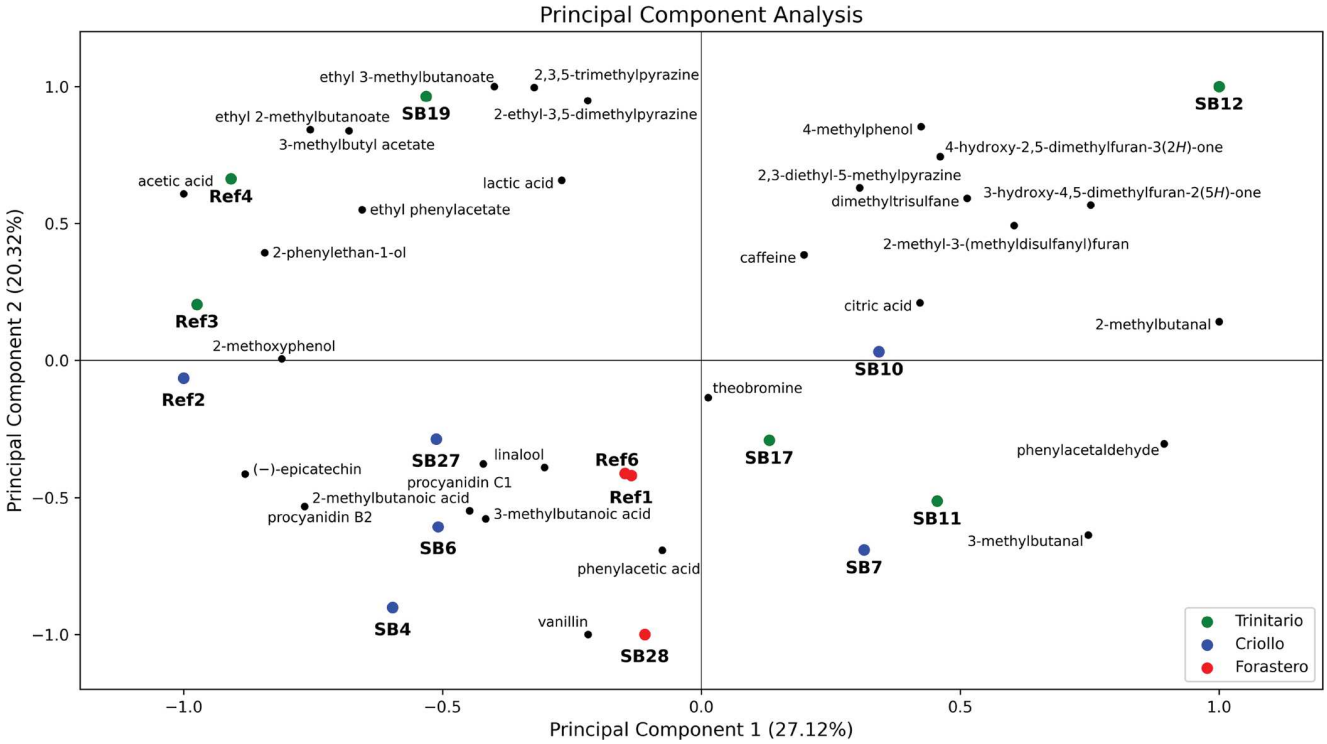

Figure 2. Principal component analysis of flavor-active compounds with DoT factors >1.

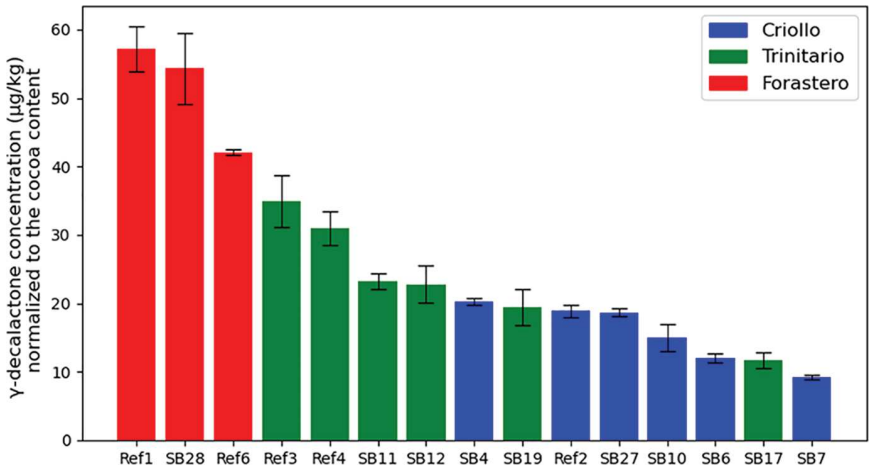

Figure 3. Concentrations of γ-decalactone normalized to the cocoa content in the analyzed chocolates.

The DoT factors of the two lactones quantitated in this study, namely, γ-decalactone and γ-nonalactone, were <1 in all samples. However, the concentrations of γ-decalactone were clearly higher in the Forastero samples than in all other samples (Figure 3). Although the differences were less pronounced for γ-nonalactone with only Ref1 showing a significantly higher concentration than all other samples, additive effects, maybe with contributions of further lactones, could eventually lead to an impact of lactones on the overall flavor. Notably, Frauendorfer and Schieberle observed higher concentrations of a δ-octenolactone in Forastero cocoa beans than in Criollo cocoa beans.[25] In a recent investigation, the coconut-like smelling (*R*)-δ-2-decenolactone was shown to have the highest flavor impact within the group of lactones in cocoa samples with pronounced coconut odors.[33] However, it is not clarified yet if this lactone shows higher concentrations in Forastero chocolates similar to γ-decalactone and γ-nonalactone. (*R*)-δ-2-Decenolactone should therefore be additionally considered in further studies.

The similarities within the Forastero chocolates were not limited to the odor-active compounds but were as well observed among the taste-active compounds. The DoT factor of caffeine in the Forastero chocolates was clearly the lowest, which was in line with other studies.[19] However, the DoT factors of theobromine differed not significantly between the varieties, and theobromine was assumed to have the highest impact on the bitter perception according to the DoT concept.

In summary of the Forastero data, the Forastero chocolates shared many common characteristics in their flavor compound profiles in particular for the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde. Furthermore, they were characterized by high DoT factors of 2- and 3-

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methylbutanoic acid, phenylacetic acid, and linalool and by low DoT factors of caffeine and esters. The DoT factors of lactones were <1 but the high concentrations in the Forastero chocolates might influence the flavor properties via additive effects. No clear differences were found in the concentrations of cyclo(L-pro-L-val) between the Forastero chocolates and the other ones. However, a recent investigation on the 2,5-diketopiperazine compositions of 33 chocolates made of different bean varieties showed that Forastero chocolates are characterized by high concentrations of 2,5-diketopiperazines including cyclo(L-pro-L-val).[27] The combination of different 2,5-diketopiperazines may enhance the bitter taste of chocolate by additive effects.

Flavor-Active Compounds in the Trinitario Chocolates. The heat map (Figure 1) and the PCA (Figure 2) indicated a higher variability in the flavor compound compositions in the Trinitario chocolates than in the Forastero chocolates. However, similarities were as well observed in the Trinitario group. Three of the six Trinitario chocolates—Ref3, Ref4, and SB19—were linked closely together in both, the heat map and the PCA plot. Their low values for PC 1 and high values for PC 2 were associated with high DoT factors of esters and acetic acid. The heat map illustrated the high DoT factors of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, and ethyl phenylacetate in the samples. High DoT factors of the esters and acetic acid were in line with a fruity and acidic flavor profile. Ref3 and Ref4 were both described as distinctly fruity and acidic in our previous publication.[26] With 13.2, 7.47, and 6.20, SB19 showed even higher DoT factors of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and 3-methylbutyl acetate than Ref3 and Ref4 (Figure 1). The DoT factor of acetic acid in SB19 was 9390, and the second highest among all samples. The three chocolates Ref3, Ref4, and SB19 showed the expected flavor compound profiles as Trinitario beans are known for their fruity aroma and high concentrations of acetic acid.[4,16] Trinitario liquors were clearly differentiated from Forastero liquor by their fruity, acidic, and floral sensory characteristics in another study.[11]

Even though the three Trinitario chocolates Ref3, Ref4, and SB19 were close in the PCA plot, there was no separation of the complete Trinitario group from the other samples. High DoT factors of esters and acetic acid were not observed in the other three Trinitario chocolates SB17, SB11, and SB12. With 1.84 and 3390, SB11 showed the lowest DoT factors of ethyl 2-methylbutanoate and acetic acid among all samples. SB17 and SB11 were close to the three Forastero chocolates in the PCA plot. Both showed high DoT factors of the Strecker aldehydes 2-methylbutanal, 3-methylbutanal, and phenylacetaldehyde that were in the range of the Forastero chocolate DoT factors. Trinitario sample SB12 was clearly separated from all other chocolates in the PCA plot and was linked closely to the three Forastero chocolates in the heat map. Similar to the Forastero chocolates, this sample showed high DoT factors of compounds that indicate intense cocoa-like flavor notes.[26] With 524, 3280, and 181, the DoT factors of 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, dimethyltrisulfane, and 2-methylbutanal were even higher in SB12 than in the Forastero chocolates. Additionally, SB12 showed the highest DoT factors of 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 4-methylphenol, and 2,3-diethyl-5-methylpyrazine among all samples. SB12 was further characterized by the lowest DoT factors of phenylethan-1-ol, (−)-epicatechin, and procyanidin B2 among all samples. The positive scores on PC 2 of SB12 were linked to high DoT factors of the esters. With 4.40, the DoT factor of ethyl 3-methylbutanoate in SB12 was even higher than the one in Ref4, which amounted to 2.83. However, with 5.37, the DoT factor of ethyl 2-methylbutanoate was not especially high and in the range of the three Forastero chocolates.

In summary of the Trinitario data, the Trinitario chocolates shared common characteristics in their flavor compound profiles with predominantly high DoT factors of fruity smelling esters and acetic acid. Furthermore, the Trinitario chocolates showed the significantly highest DoT factors of 3-methylbutyl acetate, ethyl phenylacetate, 4-methylphenol, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, and lactic acid among all samples when averaging all values from one variety. Even though common features were observed, the Trinitario chocolates showed a high variability in their flavor compound profiles, which was clearly visible in both, the heat map and the PCA plot. The high variability might be explained by the origin of Trinitario as a hybrid between Criollo and Forastero.[3] The flavor compound profiles might vary depending on whether the Criollo or Forastero background is dominating as already suggested for the 2,5-diketopiperazine concentrations in a previous study.[27] The similarities in the flavor compound profiles of SB17 and SB11 to the ones of the Forastero chocolates could consequently result from a dominating Forastero background.

Flavor-Active Compounds in the Criollo Chocolates. The Criollo samples showed a high variability in the flavor compound composition in the heat map (Figure 1). The PCA (Figure 2) indicated a less pronounced variability in the Criollo samples than in the Trinitario samples. Ref2 (Criollo) was linked to the three Trinitario chocolates Ref3, Ref4, and SB19 in the heat map as well as in the PCA. With 10.9, 4.64, and 9030, Ref2 showed comparably high DoT factors of ethyl 2-methylbutanoate, ethyl 3-methylbutanoate, and acetic acid similar to the three Trinitario chocolates. This was in line with a fruity flavor profile of Ref2.[26] Consequently, flavor compound profiles with high DoT factors of fruity smelling esters and acetic acid were not exclusive to chocolates made from Trinitario beans. In contrast, the two Criollo chocolates SB7 and SB10 showed very low DoT factors of the esters and acetic acid. Thus, the two chocolates had similar characteristics to the Trinitario chocolates SB11 and SB17 and the three Forastero chocolates. In addition, they showed relatively high DoT factors of the Strecker aldehydes 2-methylbutanal and 3-methylbutanal and the highest DoT factors of phenylacetaldehyde among all samples. With 1440, the highest DoT factor of 3-methylbutanal was found in SB7. The further three Criollo chocolates SB4, SB6, and SB27 did not show especially high or low DoT factors of certain compounds. All Criollo chocolates showed lower DoT factors of dimethyltrisulfane and citric acid than the other chocolates. The unique aroma profiles of the analyzed Criollo chocolates may be caused by the specific combination of odorants rather than by high DoT factors of individual odorants. The Criollo chocolates shared common features also in other taste-active compounds. Except for SB7, the Criollo chocolates showed mostly higher DoT factors of (−)-epicatechin, procyanidin B2, and procyanidin C1 than the Forastero and Trinitario chocolates. High concentrations of procyanidins in Criollo liquors were also reported by Counet et al. and linked to the short fermentation time of the Criollo beans.[15]

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Flavor-Active Compounds in the Nacional Chocolate. The Nacional chocolate (Ref5) showed typical characteristics known from the literature[19,9] like high DoT factors of (−)-epicatechin, procyanidin B2, and procyanidin C1. Furthermore, Ref5 was characterized by distinct floral notes in our previous investigation,[26] which is considered characteristic for Nacional cocoa.[3] As discussed previously,[26] 2-phenylethan-1-ol was assumed to be mainly responsible for the floral notes in this sample and high concentrations of this compound in Nacional samples were also reported in other studies.[19,34] Within the new sample set of 16 chocolates considered in the current study, the DoT factor of 2-phenylethan-1-ol was outstanding in Ref5 together with the Trinitario sample Ref3. In contrast to the Nacional sample, Ref3 was further characterized by high DoT factors of esters. Furthermore, the Nacional chocolate showed the lowest DoT factors of 3-methylbutanal, dimethyltrisulfane, 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one, 2-methyl-3-(methyldisulfanyl)furan, and 2-ethyl-3,5-dimethylpyrazine among all samples and the DoT factor of 2,3,5-trimethylpyrazine was <1. Likewise, Tuenter et al.[19] found significantly lower concentrations of 2-methylbutanal, 3-methylbutanal, and pyrazines in a Nacional liquor than in a West African blend liquor. High concentrations of linalool as described for Nacional cocoa beans in the literature[28] were not confirmed in our current study. With 48.8, the DoT factor of linalool in the Nacional chocolate was not especially high. For example, all Forastero chocolates showed higher DoT factors with a top value of 175 in Ref1. Further Nacional samples should be analyzed to confirm these observations especially concerning the DoT factor of linalool.

Influence of the Cocoa Bean Variety on the Flavor Compound Compositions of Dark Chocolates. Common characteristics were observed for chocolates made from the same variety in particular for the Forastero and Trinitario chocolates. However, the Trinitario and Criollo chocolates showed wide variations in their flavor compound profiles. Wide variations in the volatile composition of Criollo and Trinitario cocoa were as well observed by Utrilla-Vázquez et al.[17] They compared three Criollo and two Trinitario cocoas at different processing stages, namely, fresh, fermented, and dried, and found no clear differences between the volatile compositions of Criollo and Trinitario beans. Instead, each analyzed cocoa sample showed its specific composition. Even though the pyrazine concentrations were predominantly higher in the Forastero liquors, Counet et al.[15] found even higher concentrations of pyrazines and dimethyltrisulfane in a Criollo liquor. They assumed higher concentrations of precursors formed during the long fermentation time in this sample. This was similar to our observation that high DoT factors of compounds that were characteristic for the Forastero chocolates were found in samples from other varieties as well. Utrilla-Vázquez et al.[17] further reported a greater influence of the process than of the cocoa bean variety. This observation was in agreement with another study.[13] Apart from the fermentation, growing conditions and other cocoa processing steps have a strong influence on the flavor profile.[4,9,10,31] The flavor quality of chocolate depends on the fermentation time of the cocoa beans[35] and roasting influences the formation of compounds that are important for cocoa-like flavor notes such as Strecker aldehydes, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyltrisulfane.[24,25] Different roasting conditions[36] and conching parameters[37] further influence the concentrations of flavor-active compounds. The high influence of the processing conditions has to be considered when analyzing fully processed chocolates. Varying processing conditions may partly explain the wide variations in the Trinitario and Criollo chocolates. Usually, processing conditions are adapted to the cocoa bean quality to obtain the desired flavor profile. For example, Forastero beans are usually fermented for a longer period than Criollo beans[10,31] and different roasting protocols are recommended for the varieties Forastero, Trinitario, and ancient Criollo types.[32] The observed similarities in the flavor compound profiles of the chocolates made from one variety could therefore partly result from similar processing conditions.

In summary, common features were observed for chocolates made from the same cocoa bean variety, which indicated a partial influence of the variety on the flavor compound profiles of dark chocolates. The six Trinitario chocolates were predominantly characterized by high DoT factors of acetic acid and fruity smelling esters with especially high values in three of the six Trinitario chocolates. The three Forastero chocolates showed common features regarding the DoT factors of compounds such as 2- and 3-methylbutanal, 2- and 3-methylbutanoic acid, phenylacetic acid, and caffeine. Flavor compound profiles similar to the ones of the Forastero chocolates with high DoT factors of 2- and 3-methylbutanal, dimethyltrisulfane, and 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one were observed in Trinitario and Criollo chocolates. Flavor compound profiles characterized by high DoT factors of esters and acetic acid were not only found in Trinitario chocolates but also in a Criollo chocolate. Consequently, it can be assumed that these flavor characteristics are not exclusive to a single variety. Even though high DoT factors of certain compounds seemed to be characteristic of certain varieties, the flavor properties of dark chocolates are defined by the whole flavor compound composition. It can be assumed that the unique flavor profiles of single-variety dark chocolates are caused by specific combinations of all flavor-active compounds. The chocolates made from Criollo and Trinitario beans showed a high variability in their flavor compound profiles, which suggested that the variety alone is not determinant for a specific flavor profile. The impact of both, the genetic background and the processing, seemed to be reflected in the flavor compound compositions of the chocolates in this study. To conclude, our study clearly demonstrated that single-variety dark chocolates from fine or flavor cocoa show high variability in their flavor compound profiles. This flavor diversity could at least be partly linked to the cocoa bean variety.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.2c00418.

Roasting parameters of the small-batch chocolates; concentrations of selected odorants and tastants in the small-batch chocolates as means of triplicates; mean concentrations normalized to the cocoa content; and mean dose-over-threshold factors calculated from concentrations normalized to the cocoa content (PDF)

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Notes

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ABBREVIATIONS

DoT, dose over threshold; GC−MS, gas chromatography−mass spectrometry; PC, principal component; PCA, principal component analysis; 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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8.3.3 Summary and individual contributions

In recent years, the share of fine flavor cocoa and the production of small batch bean-to-bar chocolates increased rapidly. Such chocolates are made from cocoa of a defined variety and origin and are characterized by their unique flavor profiles. Although flavor is the main criterion for discriminating fine flavor cocoa from bulk cocoa, other indicators are often used in practice. Genetic variety is one of these indicators, and flavor quality is often linked to variety in the literature. However, the influence of cocoa bean variety on the flavor compound composition of chocolate has not yet been extensively studied and was therefore investigated in this study. Selected odorants and tastants were quantitated in 16 single variety dark chocolates and the DoT factors of flavor-active compounds were compared. The sample set included the six reference chocolates from the previous study (c.f. section 8.2) and ten additional commercial small batch chocolates. The three Forastero chocolates showed predominantly higher DoT factors of phenylacetic acid, 2-methylbutanoic acid, 3-methylbutanoic acid, and linalool, but lower DoT factors of caffeine than the chocolates made from other varieties. Furthermore, the Forastero chocolates had high DoT factors of compounds characteristic for cocoa-like flavor notes in common. These compounds included 2-methylbutanal, 3-methylbutanal, phenylacetaldehyde, 4-hydroxy-2,5-dimethylfuran-3(2*H*)-one, and dimethyltrisulfane. However, these characteristics were not exclusive to the Forastero chocolates and high DoT factors of the mentioned compounds were also observed in chocolates made from other varieties. The Trinitario and Criollo chocolates showed higher variability in their flavor compound compositions than the Forastero chocolates. The Criollo chocolates showed predominantly high DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1. Similarities in the Trinitario chocolates were high DoT factors of esters and acetic acid with three of them showing especially high values. One Criollo chocolate also showed high DoT factors of fruity smelling esters and acetic acid, demonstrating that these characteristics were not unique to Trinitario chocolates. Two Trinitario chocolates and two Criollo chocolates showed similar characteristics to the Forastero chocolates, such as high DoT factors of Strecker aldehydes. The Nacional chocolate showed even higher DoT factors of (–)-epicatechin, procyanidin B2, and procyanidin C1 than the Criollo chocolates and a high DoT factor of 2-phenylethan-1-ol. The high variability in the flavor compound compositions of Trinitario and Criollo chocolates demonstrated that the genetic variety alone is not determinant for a specific flavor profile. Nevertheless, the results of this study indicated that the molecular flavor diversity of single variety dark chocolates is at least partly influenced by the cocoa bean variety.

Lisa Ullrich designed and performed the experiments, including sample work-up, quantitation, and calculation of DoT factors. She further summarized, statistically evaluated, illustrated, and interpreted the data, and prepared the manuscript. Bettina Casty designed and performed the experiments together with Lisa Ullrich, and also evaluated the data. Amandine André performed the HPLC measurements, evaluated the data, and revised the manuscript. Tilo Hühn participated in the discussion of the results. Martin Steinhaus designed the experiments, interpreted the results, and revised the manuscript. Irene Chetschik designed and supervised the experiments. She further interpreted the data, assisted in preparing the manuscript, revised it, and submitted it.

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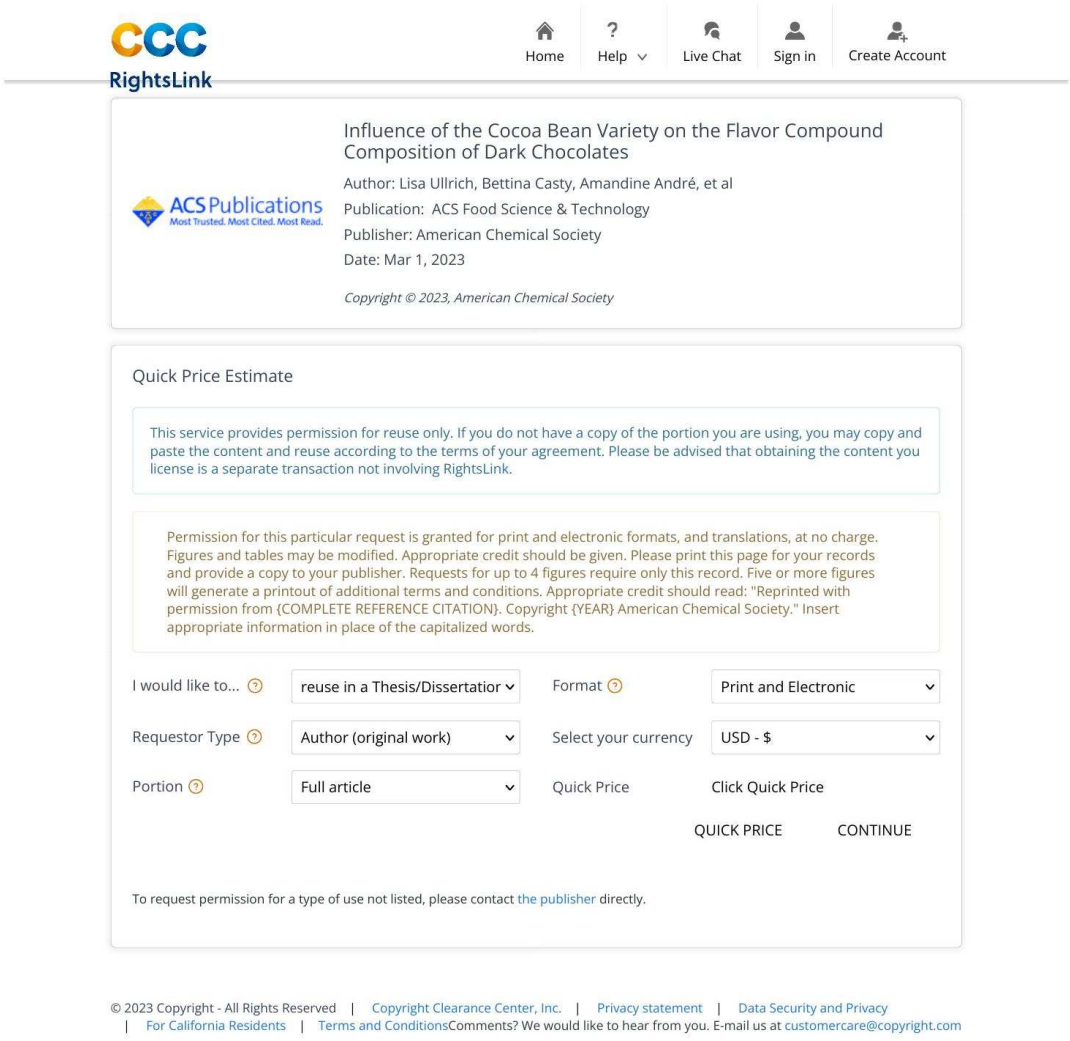

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Influence of the Cocoa Bean Variety on the Flavor Compound Composition of Dark Chocolates

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8.4 List of publications and presentations

Publications

Publications in peer-reviewed journals included in this thesis

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. https://doi.org/10.1021/acs.jafc.1c02287

Ullrich, L.; Casty, B.; André, A.; Hühn, T.; Steinhaus, M.; Chetschik, I. Decoding the fine flavor properties of dark chocolates. *J. Agric. Food Chem.* **2022**, *70,* 13730–13740. https://doi.org/10.1021/acs.jafc.2c04166

Ullrich, L.; Casty, B.; André, A.; Hühn, T.; Chetschik, I.; Steinhaus, M. Influence of the cocoa bean variety on the flavor compound composition of dark chocolates. *ACS Food Sci. Technol.* **2023**, *3,* 470–477. https://doi.org/10.1021/acsfoodscitech.2c00418

Other publications in peer-reviewed journals

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**, *8,* e10770. https://doi.org/10.1016/j.heliyon.2022.e10770

Ullrich, L.; Gillich, E.; André, A.; Panarese, S.; Imhaus, A. F.; Fieseler, L.; Chetschik, I. Influence of ozone treatment during storage on odour-active compounds, total titratable acidity, and ascorbic acid in oranges and bananas. *Applied Sciences* **2023**, *13*, 10885. https://doi.org/10.3390/app131910885

Presentations

Oral presentations at international scientific meetings

Ullrich, L.; Neiens, S.; Chatelain, K.; Kneubühl, M.; Hühn, T.; Steinhaus, M.; Chetschik, I. Release of important chocolate odorants by water: comparison of traditionally manufactured chocolate to chocolate manufactured by a novel processing technology. International Chemical Congress of Pacific Basin Societies (Pacifichem). Online, December 21, 2021.

Ullrich, L.; Casty, B.; André, A.; Hühn, T.; Steinhaus, M.; Chetschik, I. Decoding the fine flavour properties of dark chocolates. International Symposium on Cocoa Research (ISCR). Montpellier, France, December 7, 2022.

Other oral presentations

Ullrich, L.; Neiens, S.; Hühn, T.; Steinhaus, M.; Chetschik, I. Impact of water on odor-active compounds in cocoa beans and chocolate. Forschungsseminar Food Chemistry and Food Quality, organized by the TUM School of Life Sciences. Online, November 23, 2020.

Ullrich, L.; Neiens, S.; Hühn, T.; Steinhaus, M.; Chetschik, I. Impact of water on odor-active compounds in cocoa. F&E-Kolloquium, organized by the ZHAW Institute of Food and Beverage Innovation. Online, November 23, 2020.

Ullrich, L.; Casty, B.; André, A.; Hühn, T.; Steinhaus, M.; Chetschik, I. Decoding the fine flavour properties of dark chocolates. F&E-Kolloquium, organized by the ZHAW Institute of Food and Beverage Innovation. Wädenswil, Switzerland, February 6, 2023.