Journal of Applied Botany and Food Quality 93, 266 - 275 (2020), DOI:10.5073/JABFQ.2020.093.033

[1]Rausch GmbH, Cocoa and Research, Berlin, Germany
[2]Biocenter Klein Flottbek and Botanical Garden (BioZ Flottbek), University of Hamburg, Germany
[3]Organic Farming with focus on Sustainable Soil Use, Institute of Crop Science and Breeding II, Justus-Liebig University Giessen, Germany
[4]Cacao Plus Internacional, Costa Rica
[5]Crop Plant Museum Gorleben, CPMG, Germany

Besides variety, also season and ripening stage have a major influence on fruit pulp aroma of cacao (*Theobroma cacao* L.)

E. Hegmann[1,2]*, W. Niether[3], W. Phillips[4], C. Rohsius[1], R. Lieberei[2,5]

(Submitted: October 16, 2020; Accepted: November 27, 2020)

Summary

More than 1000 different cacao varieties are described. Only few are considered as fine or flavour cocoas, meaning they have the potential to develop special flavour characteristics after appropriate fermentation and drying. It is assumed that aroma compounds located in the fruit pulp migrate into the seed during fermentation. We studied the fine aroma potential of five cacao varieties selected at CATIE, Costa Rica, by analysing aroma compounds in their fresh fruit pulps using Headspace SPME-GCMS. Pulps of unripe, ripe and overripe fruits harvested in the dry and rainy season, respectively, were compared to the control genotypes EET 62 and SCA-6, both known for high amounts of fine aromas described as e.g. "fruity", "floral" or "spicy". All genotypes contained a basic content of the two dominating esters 2-pentanol acetate and 2-heptanol acetate, combined with a mixture of aroma-active compounds with small peak areas that form the variety-specific aroma character. Total aroma diversity and intensity increased during ripening. Aroma profiles were more diverse when fruits ripened during the dry season, whereas aroma intensity was higher in the rainy season. Thus, the fruit and environmental conditions prior to harvest can already play a decisive role for the aroma potential of the cacao pulp. Due to their aroma profiles, the varieties from CATIE can be classified as fine or flavour cocoas.

Keywords: cocoa; flavour; fine or flavour cocoa; secondary plant metabolites

Introduction

The seeds of the cacao tree (*Theobroma cacao* L.) are the key raw material for chocolate products. They are the source of income for millions of small-scale farmers in tropical and subtropical countries. Industry and trade distinguish between bulk cocoa and fine or flavour cocoa. Whereas bulk cocoas are characterised by a broad chocolate flavour that builds up during the fermentation and drying process and usually lack any special flavour attributes, fine cacao varieties additionally develop special aromas described for example as fruity, floral or spicy (Sukha, 2008) that originate from plant secondary metabolites.

The most important volatile and non-volatile fine aroma compounds in cocoa belong to the groups of terpenes, alcohols, aldehydes, carboxylic acids, methyl ketones and esters (Aprotosoaie et al., 2016; Ziegleder, 1990; Eskes et al., 2007; Kadow et al., 2013). They derive from different synthesis pathways, differ in their chemical properties and undergo transformation during cocoa post-harvest processes (Belitz, Grosch and Schieberle, 2009). In addition to these compounds, pyrazines and furan derivatives play a key role in the flavour of chocolate liquors as they display e.g. nutty notes and enhance the overall flavour (Afoakwa et al., 2008; Aprotosoaie et al., 2016; Belitz, Grosch and Schieberle, 2009; Rodriguez-Campos et al., 2011; Schieberle and Pfnuer, 1999).

Fine or flavour aroma compounds can derive from different tissues within the seeds or are a result of yeast and bacterial activity during fermentation (Schwan and Wheals, 2004), but most of them are formed or stored in the fruit pulp (Eskes et al., 2007; Pino et al., 2010). Aldehydes for example can be found in the fresh fruit pulp, but are also built within the seeds during the fermentation process in the course of Strecker degradation from amino acids (Belitz, Grosch and Schieberle, 2009; Afoakwa et al., 2008). Also, they derive from primary alcohols via autoxidation or enzymatic lipid peroxidation of saturated and unsaturated fatty acids. However, due to the fatty acid pattern of the cacao seeds (with a ratio of palmitic, stearic, oleic and linoleic acid of 25:37:34:3, respectively) the autoxidation process can be largely excluded (Belitz, Grosch and Schieberle, 2009). Secondary alcohols like 2-nonanol can be oxidized to ketones and reduced to esters under the influence of carboxylic acids.

With some rare exceptions (e.g. ancient Criollos) most fresh cacao seeds are inedible, they must first be fermented and dried to reduce bitterness and astringency. Cacao seeds are usually fermented spontaneously in their own mucilaginous fruit pulp. Yeasts and bacteria utilize sugars from the pulp for their metabolism and enable molecules from the pulp to enter the cacao cotyledons during the fermentation process (Schwan and Wheals, 2004). Chetschik et al. (2018) found the same aroma compounds in the cacao fruit pulp and in the cocoa beans: since many aroma compounds are lipophilic, it can be assumed that these substances are transferred from the cacao pulp to the cotyledons and accumulated in storage fats (Kadow et al., 2013). Depending on variety, season and region, cacao seeds contain between 45% and 60% fat (Pires et al., 1998; Lockwood and Eskes, 1996; Rohsius, 2010), so that large quantities of the less polar aromatic substances from the pulps could be stored in the lipid vacuoles of the mesophyll cells after fermentation (Rohsius, 2007). Cacao fruit pulp may therefore play a crucial role as a precursor in the aroma development, composition and intensity of the cocoa beans and ultimately has the potential to contribute to the organoleptic character of the chocolate liquor.

Fruit pulp is a combination of modified mesocarp and endocarp and develops during the ripening process of the cacao fruit (Lieberei and Reisdorff, 2012; Andersson et al., 2006). During the last weeks of fruit ripening, the pulp begins to accumulate sugars and acids while the content of pectin decreases (Rohsius, 2007). Due to biochemical changes within the fruit the pulp becomes more viscous and softer towards the end of fruit ripening. Even when ripe, cacao fruits remain on the tree and do not fall off (Rohsius, 2007). Since ripe fruits can only be distinguished from unripe fruits by their colour and the

* Corresponding author

cacao tree produces fruits all year around (see PHILLIPS-MORA et al., 2013, Fig. 13), knowledge and proper harvest management are indispensable. Nonetheless, cacao farmers often harvest unripe or already overripe fruits and combine and process all beans together. This may influence the overall aroma of the fruit pulp in the fermented cocoa mass and has an effect on the final cocoa bean aroma. Drought increases the aroma concentration in many fresh fruits like wine grapes (DELUC et al., 2009; SAVOI et al., 2016) and its processed products like wine (SAVOI et al., 2020). An effect of the seasons on the aroma of cocoa beans or cacao fruit pulp has not yet been studied, though several studies show an effect of environmental conditions and season on the molecular composition of cocoa beans (NIETHER et al., 2017; DE ARAUJO et al., 2018; SUKHA, 2008).

Here, we studied the aroma potential of five cacao varieties (CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58 and ICS-95 (T1)) from CATIE, Costa Rica (PHILLIPS-MORA et al., 2013) by analysing the aroma characteristics of the fresh fruit pulps. These clones are the first materials accessible to farmers which possess, to a greater or lesser degree, a combination of desirable characteristics, particularly resistance to frosty pod (*Moniliophthora roreri*), yield potential and flavour quality (PHILLIPS et al., 2009; PHILLIPS-MORA et al., 2013). They are now widely distributed in Central America and Mexico and are being evaluated for cultivation in Brazil (PHILLIPS-MORA et al., 2017).

Unripe, ripe and overripe fruits were harvested in the dry and in the rainy season to analyse the influence of the ripening stages and the effect of climatic conditions during fruit ripening on aroma volatiles in the pulp. The intensity and diversity of aroma compounds were compared to those detected in the two control genotypes EET 62 ("Nacional" cacao, according to DELGADO et al., 2003) and SCA-6 ("Contamana" cacao, according to MOTAMAYOR et al., 2008) that are known for their fine aroma notes (KADOW et al., 2013).

We hypothesize that (1) the cacao varieties from CATIE have the potential to develop a diversity of aroma compounds due to their genetic background, (2) intensity and composition of different aroma substances increase during fruit ripening and (3) pulp aroma intensity is enhanced in fruits of the dry season as shown for other fruit crops.

Material and Methods

Study location and fruit sampling

Fruit sampling of the potential fine or flavour varieties (CATIE-R1, CATIE-R4, CATIE-R6, ICS-95 (T1), PMCT-58) and the varieties used as control genotypes (EET 62, SCA-6) was conducted at the International Cacao Collection (IC3) of the Tropical Agricultural Research and Higher Education Center (CATIE). The institute is located in Turrialba, in the central highland of Costa Rica (602 m a.s.l.) with a mean annual precipitation of 2645 mm and a mean temperature of 22.5 °C (ARCINIEGAS, 2005). From all varieties, up to three unripe, ripe and overripe fruits, respectively, were harvested each in November 2012 (ripening during the rainy season) and in April 2013 (ripening during the dry season). Fruit ripening stages were determined visually by the typical fruit colour characteristics of each variety and their changes during ripening (PHILLIPS-MORA et al., 2013). Due to production limitations, the number of three fruits could not be reached for all varieties, seasons and ripening stages resulting in a total of 51 samples from the rainy and 67 samples from the dry season. The correct number of fruits used for analysis is shown in the Annex Table 1A. The fruits were harvested in the morning hours, disinfected, individually wrapped in 4 cm thick foam, stored in a well isolated shipping box and immediately sent by express (3-4 days) to the Institute of Food Chemistry at the University of Hamburg, Germany, for analysis.

Analysis of volatile aroma compounds in fresh cacao fruit pulps

Cacao fruits were opened and the ripening stage of the pulps determined: pulps of unripe fruits were firmly connected with cotyledons and mesocarp of the fruit, starch degradation had not yet occurred; ripe fruit pulps had slightly dissolved from the mesocarp already and the pulps were aqueous; overripe fruits presented pulps completely separated from the mesocarp and, depending on the variety, they were of slightly brown colour. From each fruit, two fresh pulp samples of each 5 g including the testa were removed from the cotyledons using a scalpel and directly weighed into a 20 mL Headspace Crimp Neck Vial N 20 (Macherey Nagel). Vials were closed gastight using an aluminium crimp cap type N20 (8 mm, Septum: blue silicone/PTFE colourless, 3 mm, Macherey Nagel). Samples were stored at -80 °C. For the analysis, each sample vial was heated in a water bath at 30 °C for 15 min. Volatile aroma compounds accumulated in the headspace were obtained by solid-phase micro-extraction (SPME) during 15 min at 30 °C using a PDMS / DVB fibre (65 µm, needle size 24Ga, SPME Fiber Assembly, StableFlex, Supelco, Sigma-Aldrich Group). Analytes were then manually injected into the gas chromatograph (6890 Series GC-System, Agilent Technologies) and separated into the individual substances for subsequent identification and quantification in the mass spectrometer (S 5973 Network Mass Selective Detector, Agilent Technologies) (Tab. 1). Chromatograms were evaluated using OpenChrom 9.0. For aroma identification, mass spectra of the volatiles were compared with the reference spectra of the NIST library (National Institute of Standards and Technology). Fruit pulp samples were analysed in duplicate. One sample of the control genotype EET-62 indicated with 5.29E + 09 the highest mean total peak area of volatile aromas in its pulp of ripe fruits. This was used as reference area for calculating the proportional peak area of all samples. Aroma compounds detected in the fresh fruit pulps were assigned to the aroma groups "green", "herbal", "fruity", "floral", "spicy", "woody" and "earthy" (Tab. 2, according to MOSCIANO, 1989; 1990a, b; 1991a, b, c, d; 1992a, b; 1993a, b; 1995a, b, c; 1996a, b; 1997a, b; 1998; 2000; 2001a, b; 2009 via *the good scents company*, SURBURG and PANTEN (2006), NOZAKI et al. (1997), HUI (2010), LAN-PHI et al. (2009)). The difference between the sum of peaks and the total peak area was grouped as "others" together with four aroma compounds that were either not clear in their aroma characteristics or unassignable to one of the other aroma groups.

Statistical analysis

A multivariate PCA (Principal Component Analysis) was performed on 67 pulp samples from the dry season only, using all the detected aroma compounds to give a descriptive overview over the global dataset and the relation of the levels of the factors "variety" (CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58, EET 62, SCA-6) and "ripening" (unripe, ripe, overripe). A second PCA was performed from 95 pulp samples from ripe and overripe fruits (pooled) for the factors "variety" (CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58, EET 62, SCA-6) and "season" (dry, rainy). PCA plots were visualized with the *ggfortify* R package (HORIKOSHI and TANG, 2016) within the statistical programming environment R (R Core Team, 2020). Spearman's rank correlation (ϱ) for non-normally distributed data was used to evaluate bivariate relationships between the aroma groups.

Linear mixed-effects models (lmerTest, KUZNETSOVA et al., 2017) were used to study the factors influencing the aroma groups (response variables) of the fruit pulp. In the first model with samples from the dry season only, the fixed factors "variety" (CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58) and "ripening" (unripe, ripe, overripe) and their interaction were analysed. In a second model using only ripe and overripe (pooled) fruits from both seasons, the effects of the fixed factors "variety" (CATIE-R1, CATIE-R4, CATIE-R6,

Tab. 1: GCMS structure and conditions

Gas chromatograph 6890plus

Injector	KAS 4			
Program	Start-Temp.: 200 °C, hold for 30 s; 12 °C min^{-1} at 240 °C, hold for 10 min			
Front Inlet	Mode:	pulsed splitless		
	pressure:	48,5 kPa		
	pulse pressure:	250 kPa		
	pulse time:	30 s		
	purge flow:	40.1 mL min^{-1}		
	purge time:	28,8 s		
Column	DB-WAX (Agilent J&W, 30 m, 0,25 mm inner diameter, 0,25 µm, Catalogue-No.122-7032)			
Carrier gas	Helium 4,6			
Flow	1 mL min^{-1}; constant			
Oven	Temperature program:			
		Rate [°C min^{-1}]	**Temperature [°C]**	**Hold [min]**
	Initial	-	40	3
	Ramp 1	3	100	0
	Ramp 2	10	150	0
	Ramp 3	15	240	5

Mass-spectrometer S 5973

Transferline	300 °C
MS Source	230 °C
MS Quad	150 °C
Mass scan	40-400
Solvent Delay	0.5 min

ICS-95, PMCT-58) and "season" (dry and rainy) and their interaction on the aroma groups as response variables were analysed. The number of pulp analyses per treatment combination (replication, four levels) was added as a random factor in both models and "ripening" (ripe and overripe) was added as a random factor in the second model. When statistical differences were found, a *post-hoc* pair comparison of least significant means (*lsmeans* R package, LENTH, 2016) was applied. When necessary, data were Box Cox-transformed to meet the normality and homoscedasticity requirements. Data are shown as mean with the standard error of means. Data frames were managed with the *plyr* R package (WICKHAM, 2011). Graphs were designed with the *ggplot2* R package (WICKHAM, 2016).

Results

In total, we detected 56 aroma compounds in the fresh fruit pulps out of eight aroma groups (Tab. 2). Not all volatiles were found in all varieties. We detected 22 and 26 aroma compounds in EET 62 and SCA-6, respectively. In the CATIE varieties, many of these aroma compounds were detected in addition to others summing up to a total of 35 aroma compounds in CATIE-R1, 44 in CATIE-R4, 37 in CATIE-R6, 33 in PMCT-58 and 41 in ICS-95.

The evaluation of the GCMS chromatograms of both seasons showed that EET 62 had the largest mean total peak area (5.29 E+09), followed by ICS-95 (3.89 E+09) and SCA-6 (3.73 E+09). The varieties CATIE-R4 (2.91 E+09), CATIE-R1 (2.99 E+09) and CATIE-R6 (2.99 E+09) showed similar mean total peak areas. The genotype PMCT-58 presented the smallest mean total peak area with 2.46 E+09, meaning less than half of EET 62. Across all varieties, we observed an effect of the season on the expression of aroma compounds: aroma profiles were more diverse in fruit pulps from the dry season whereas aroma intensity (greater mean peak areas) was higher in samples from the rainy season (Tab. 2). All varieties showed many different "fruity" compounds. In comparison, the amount of detected compounds associated with the groups "woody", "earthy" and "spicy" was rather low. In ICS-95 and EET 62 the aroma group "green" dominated with 2-heptanol acetate as principal compound. The cacao varieties CATIE-R4 and CATIE-R6 showed very similar aroma compositions with dominating peaks in the aroma groups "floral", "fruity" and "spicy", such as the acyclic monoterpenes α-ocimene and α-myrcene. In addition, we detected traces of the sesquiterpene α-bergamotene ("woody") as well as the ester linalyl acetate ("herbal") in these varieties, similar to the control SCA-6. The pulp aroma of CATIE-R1 consisted primarily of alcohols, esters and ketones. The high proportion of "fruity" aromas based on 2-pentanol acetate and 2-pentanone. In PMCT-58 we found aromas that were either not present in the other varieties or only to a smaller extent, such as the "floral" aromas styrallyl alcohol, styrallyl acetate and benzacetaldeyde or the "woody" α-gurjunene (Tab. 2). PMCT-58 did not show any "earthy" compounds.

The first two components of the PCA explain 38.65% of the variance between the samples (Fig. 1). The aroma compounds were distributed in the PCA according to their aroma groups (Fig. 1A). The first axis of the PCA (PC1) was mainly loaded with compounds of the "green" (e.g. pentyl-furan and 2-octenal) and "herbal" (e.g. 3-octanone and 1-hexanol) on the positive side, on compounds of the "floral" (e.g. *trans*-linalooloxide and styrallyl-alcohol) and "fruity" groups (e.g. pentanol-acetate and *trans*-β-farnesene) on the negative side. The second component of the PCA (PC2) was loaded with compounds of the "fruity" group (e.g. 2-heptanol and 2-nonanone) on the positive side and "woody" groups (e.g. α-bergamotene and α-copaene) on the negative side.

The variety SCA-6 was separated from EET 62, PMCT 58 and ICS-95 (PC1) and could be separated from CATIE-R4 (PC2). ICS-95 and EET 62 were clustered to the positive side of PC2 (Fig. 1B), where "fruity" aroma compounds were found, only the unripe fruits of ICS-95 spread to the positive side of PC1 and the negative side of PC2, like the unripe fruits of CATIE-R4 (Fig. 1B and 1C). The different ripening stages of PMCT-58 were clustered all together. CATIE-R1 and CATIE-R6 as well as SCA-6 spread along the negative sides of PC1 and PC2.

The clusters of the ripe and overripe fruits were similar, overlapping with the areas where also "floral" and "fruity" aroma compounds were computed, while the cluster of the unripe fruits mainly overlapped with the "green", and partly with "herbal" aroma compounds (Fig. 1C).

Pulp aroma intensity and composition change throughout the fruit ripening process (Fig. 2, Tab. 3). In the pulp of unripe cacao fruits, we found less aroma diversity than in ripe and overripe fruits. All varieties except CATIE-R1 displayed very low concentrations of "floral" and "spicy" notes in unripe fruits and "woody" aroma substances were not detected in any of the cacao varieties at this ripen-

Tab. 2: Aroma compounds detected in fresh cacao fruit pulps of seven cacao varieties and their assignment to aroma groups. Studied ripening stages: 1= unripe; 2= ripe; 3= overripe. Not all ripening stages for all clones were available at both seasons. Further details in Annex Tab. 1A.

	mean peak area < 5.0E+06		mean peak area < 1.0E+08 > 1.0E+09
not detected	mean peak area < 5.0E+07		mean peak area > 1.0E+09

Aroma group	Clone	CATIE-R1		CATIE-R4		CATIE-R6		PMCT-58		ICS-95		EET 62		SCA-6	
	Season	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy	Dry	Rainy
	Ripening stage	1,2,3	3	1,2,3	3	1,2,3	3	1,2,3	2	1,2,3	1,2	2	2	2	2
	Aroma compound														
green	*trans*-2-nonenal														
	cis-6-nonenal														
	trans-2-*cis*-6-nonadienal														
	trans-2-octenal														
	trans-2-hexenal														
	cis-3-hexenyl acetate														
	2-pentyl- furan														
	2-heptanol acetate														
	hexanal														
	cis-3-hexenol														
	heptanal														
herbal	5-methyl-3-heptanone														
	2-methyl-3-buten-2-ol														
	1-octen-3-yl actetate														
	3-octanone														
	1-hexanol														
	δ-cadinene														
	trans-ocimene														
	linalyl acetate														
fruity	α-ocimene														
	benzaldehyde														
	β-cubebene														
	2-nonanone														
	2-undecanone														
	2-pentanone														
	2-octanol acetate														
	2-pentanol acetate														
	2-undecanol														
	2-heptanol														
	2-nonanol														
	octanal														
	trans-β-farnesene														
floral	linalool														
	trans-linalooloxide														
	epoxylinalool														
	benzyl acetate														
	nonanal														
	2,3-butanediol diacetate														
	benzacetaldehyde														
	styrallyl acetate														
	styrallyl alcohol														
	acetophenone														
spicy	α-myrcene														
	trans-caryophyllene														
	2-octanol														
woody	α-gurjunene														
	α-bergamotene														
	α-copaene														
earthy	*cis*-linalool oxide														
	2-octanone														
	3-octanol														
	1-octen-3-ol														
others	2-pentanol														
	1-pentanol														
	3-methyl-1-butanol														
	2-heptanone														

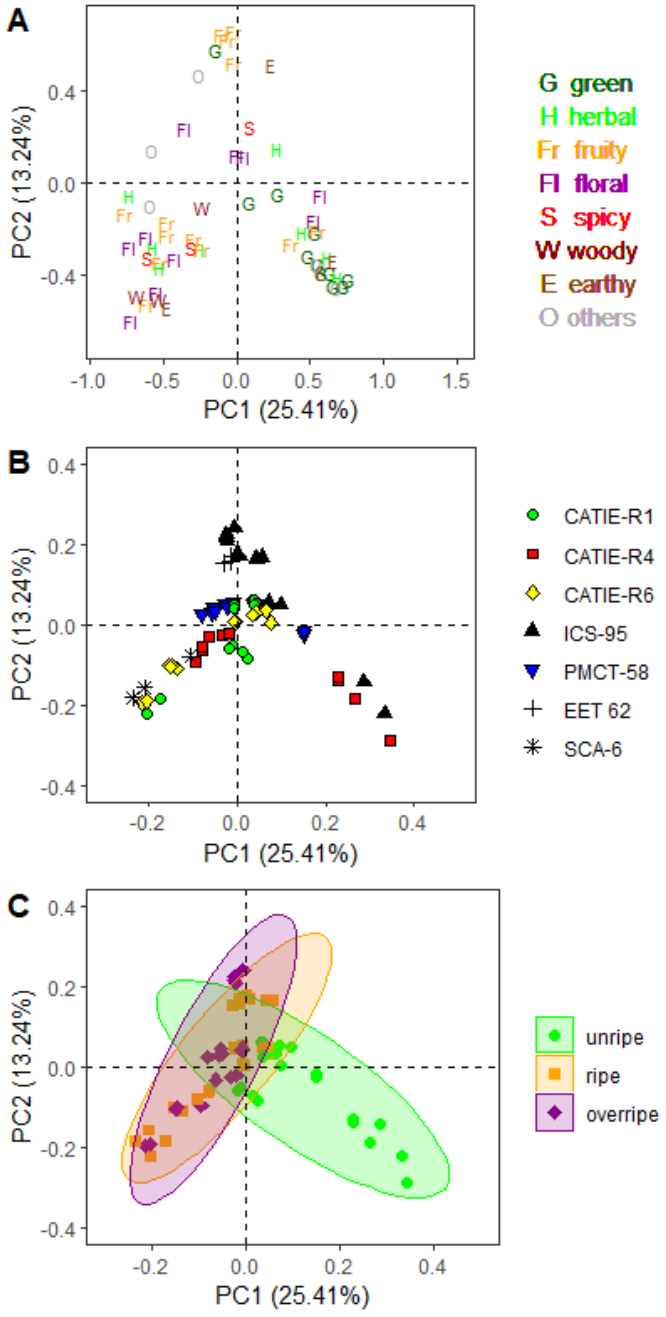

Fig. 1: **Aroma compounds in the fruit pulps of seven cacao varieties for three ripening stages (during dry season)**; A) loading of the principle component PC1 and PC2 with the aroma compounds sorted by aroma groups; B) distribution of the fruit pulp samples indicated by the factor variety C) distribution of the fruit pulp samples indicated and clustered by the factor ripening stage

ing stage. Volatiles with "fruity" attributes increased in all varieties with increasing ripening stage and were highly concentrated in pulps of overripe fruits. At this stage, ICS-95, CATIE-R4 and CATIE-R6 displayed an enhanced share of "herbal", "floral" and "spicy" aromas as well. ICS-95 stood out for its relatively high concentration of "green" components, in addition to "earthy" volatiles.

Tab. 4 shows a significant positive correlation between the aroma groups "spicy" with "floral" and "herbal". Thus, when "spicy" aromas increased, the aroma groups "herbal" and "floral" increased as well. On the contrary, the aroma groups "green" and "earthy" decreased with an increase of "fruity" volatiles. Aroma components combined in the group of "others" increased with the aroma group "green", but were negatively correlated with the aroma groups "floral" and "spicy".

Due to the overlapping of the samples from ripe and overripe fruits and the distinct pattern of the unripe samples (Fig. 1), we deleted the samples from unripe fruits and pooled the ripe and overripe samples to check the influence of the dry and the rainy season, respectively, on the aroma profile. The first two components of the PCA explain 37.66% of the variance of the dataset (Fig. 3). The positive side of PC1 is loaded with aroma compounds of the "fruity" aroma group (e.g. 2-octanol-acetate and nonanol), and the negative side is loaded with compounds of the "floral" (e.g. linalool and *trans*-linalooloxide) and "woody" aroma groups (e.g. α-bergamotene and α-copaene) (Fig. 3A). PC2 is loaded with compounds of the "fruity" (e.g. 2-nonanone and 2-heptanol) and "earthy" aroma groups (e.g. 2-octanone) on the positive side, and "floral" (e.g. benzyl-acetate and nonanal) and "woody" aroma groups (e.g. α-gurjunene) on the negative side. We observed a strong separation of the varieties ICS-95 and EET 62 from the other genotypes towards the positive side of PC1 ("fruity" aroma compounds) (Fig. 3B). PMCT-58 was clustered in the centre, as well as CATIE-R1. CATIE-R4 and CATIE-R6 stretched along PC1 directing to the "floral" and "woody" aroma groups. The samples from the dry season were separated from the rainy season (Fig. 3C). The dry season samples were mostly located at the positive side of PC1 and the negative side of PC2 ("fruity" to "floral" and "woody" aroma groups). The samples of the rainy season spread along the negative side of PC1 for the varieties CATIE-R1, CATIE-R4 and CATIE-R6 ("floral" and "woody") and to the "fruity" and "earthy" side for EET 62 and ICS-95.

All studied varieties showed higher aroma concentrations in pulps of fruits that ripened during the rainy season (Fig. 4, Tab. 5). Especially the concentration of "fruity" and "floral" aroma increased and to a lesser extend the remaining aroma groups. The difference between the season was highest in the genotypes ICS-95 and CATIE-R6. The varieties CATIE-R4 and CATIE-R6 displayed a pronounced increase in the total concentration of "spicy" and "floral" aroma towards the rainy season.

Discussion

Fine aroma potential of the cacao varieties from CATIE

The chromatograms of all studied cacao fruit pulps showed largest peak areas for the esters 2-pentanol acetate ("fruity") and 2-heptanol acetate ("green") (Tab. 2). These two esters also accounted for the largest share of total pulp aroma in comparable studies using cacao pulps from Colombian varieties (Pino et al., 2010) and EET 62, SCA-6, CCN 51 (Kadow et al., 2013), respectively. A determination or differentiation of fine or flavour cocoa from bulk cocoa by these compounds is not sufficient. The formation of variety-specific fine aromas is rather caused by a combination of compounds with smaller peak areas.

The concentrations of some minor compounds might have been below the odour threshold (Kadow et al., 2013), but in combination with other volatiles they could provide a new aroma quality through so-called "additive effects", which can be decisive for the overall aroma (Belitz, Grosch and Schieberle, 2009). The pulp aroma of SCA-6 showed high proportions of "spicy" and "floral" terpenes and alcohols and was similar to the varieties CATIE-R4 and CATIE-R6. The latter are siblings from the cross "UF 273 (T1) × PA-169" (Phillips-Mora et al., 2013) and in comparison with the other selections of CATIE, both showed highest peak areas of the monoterpenes *trans*-ocimene, α-ocimene and myrcene as well as the floral terpene-alcohol linalool. CATIE-R1 showed traces of the complex sesquiterpenes α-copaene ("woody") and *trans*-β-farnesene ("fruity"), as do CATIE-R4 and CATIE-R6. These similarities can be related to the

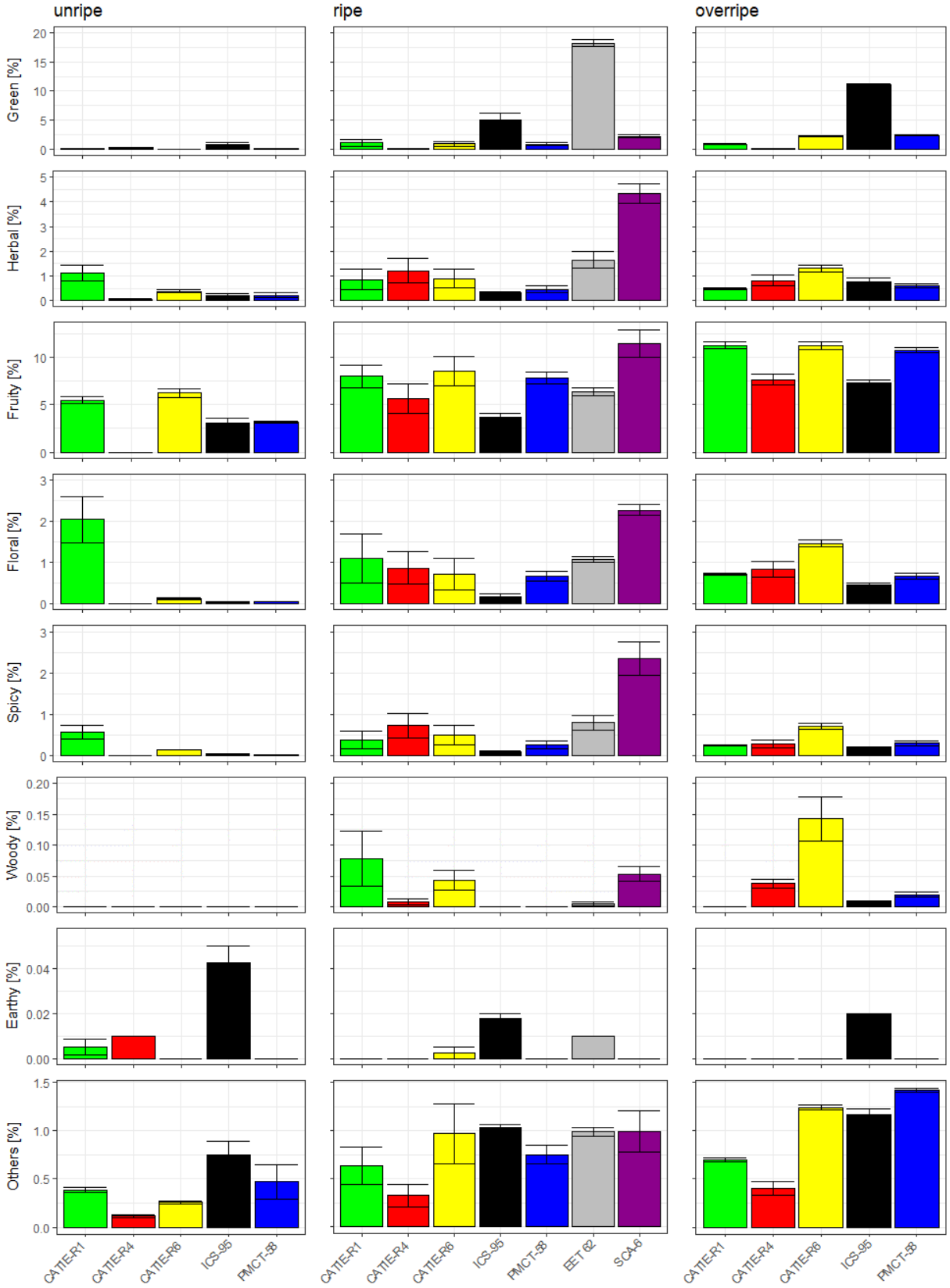

Fig. 2: Share of aroma groups detected in the cacao fruit pulps of the five varieties CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58 in three different fruit ripening stages. Shown are mean values and standard error. Maximum total peak area was detected in one sample of EET 62 and used as reference area. For comparison, we show the data of the varieties EET 62 and SCA-6 for the ripening stage "ripe".

Tab. 3: Results from linear mixed-effects models for the aroma groups and the factors variety (including the five varieties CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58), ripening stage and their interaction; F-value and level of significance: * p-value > 0.05; ** p-value > 0.01; *** p-value > 0.001; n.s. not significant

	variety F-value		ripening F-value		variety:ripening F-value	
green	47.9	***	55.8	***	7.6	***
herbal	1.7	n.s.	5.8	**	2.0	n.s.
fruity	43.5	***	121.2	***	12.5	***
floral	6.7	***	19.6	***	5.7	***
spicy	4.6	**	17.2	***	5.5	***
woody	4.6	**	3.2	n.s.	4.7	***
earthy	31.1	***	9.3	***	6.2	***
others	25.1	***	33.2	***	1.8	n.s.

fact that all three varieties have the same genetic mother UF-273 (T1) (PHILLIPS-MORA et al., 2013). UF-273 (T1) is a hybrid between the "Nacional" variety from Ecuador (63%) and the "Matina" Forastero variety from Costa Rica (32%) (PHILLIPS-MORA et al., 2017; MATA-QUIRÓS et al., 2017). Since floral aroma is a distinctive feature of the "Nacional" variety, this is possibly the origin of this trait in the three CATIE-R clones. According to ANDERSSON et al. (2006), properties of the pulp are formed from the tissue of the mother plant (mesocarp and endocarp). It can therefore be assumed that the aroma profile of the fruit pulp is principally determined by the maternal genes and the characteristics encoded by them. The potential of the varieties from CATIE to build up these aroma compounds in the fruit pulp allows a classification as fine or flavour cocoas.

Ripe cacao fruits can develop a broader aroma spectrum

We showed for the first time analytically, that aroma active-compounds with small peak areas vary greatly depending on the ripening stage of the fruit. During the fruit ripening process, the complexity of aroma compounds increases significantly. The proportion of "herbal" and "green" substances decreases in favour of "spicy", "fruity" and "floral" compounds. It must be assumed that the initial amount of pulp derived aromatic compounds is crucial for the fermentation outcome. Findings from AMORES (2006, cited in VOIGT and LIEBEREI, 2014) who observed that the floral terpene alcohol linalool appears in the cotyledons on the 3rd day of fermentation suggest that migration of these substances occur from the pulp into the cotyledon. Also ESKES et al. (2009) showed that aromas migrate from the outer part into the seed by adding aromatic fruit pulp of *Theobroma grandiflorum* and *Annona muricata* to the fermentation of cacao seeds. As a result, these aromatic compounds were perceived sensorially in the chocolate made from it. Thus, cacao fruits should be fully ripe when harvested in order to exploit the entire potential of volatile fine aroma

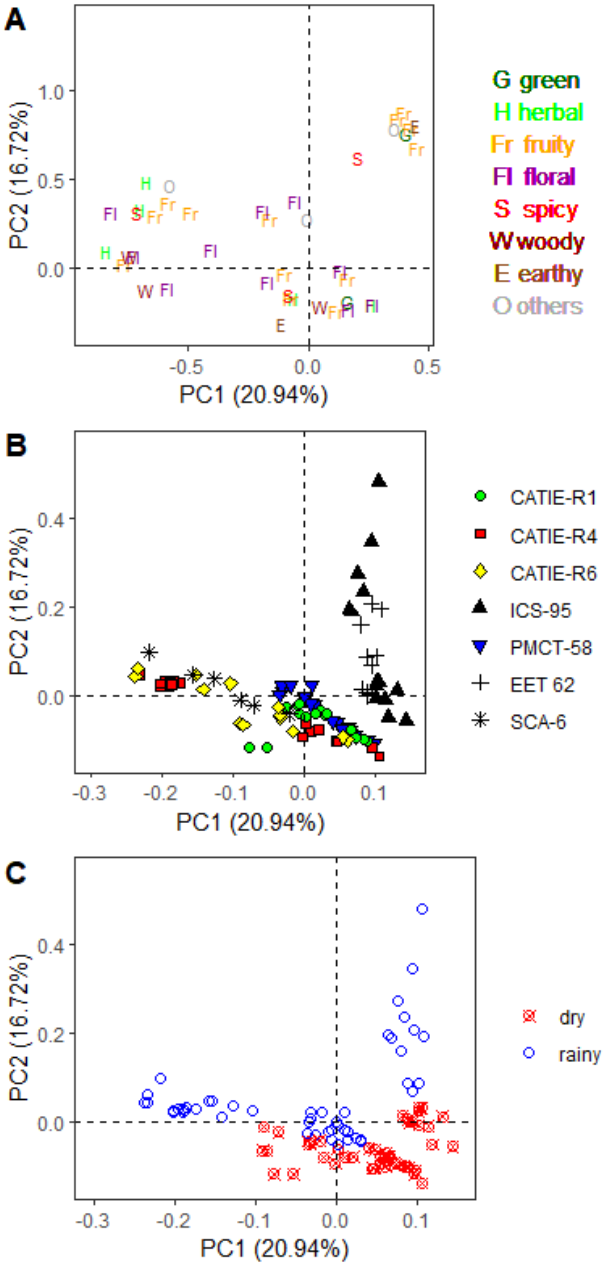

Fig. 3: Principal Component Analysis (PCA) over the aroma compounds detected in all the cacao fruit pulp samples (dry season). A) loading of the principle component PC1 and PC2 with the aroma compounds sorted by aroma groups; B) distribution of the fruit pulp samples indicated by the factor variety: C) distribution of the fruit pulp samples indicated and clustered by the factor season.

Tab. 4: Aroma groups. Correlation matrix of the aroma groups with the correlation coefficient ϱ and the level of significance (* p-value > 0.05; ** p-value > 0.01; *** p-value > 0.001; n.s. not significant)

	green		herbal		fruity		floral		spicy		woody		earthy	
herbal	-0.21	n.s.												
fruity	-0.70	*	-0.38	n.s.										
floral	-0.35	n.s.	0.38	n.s.	0.00	n.s.								
spicy	-0.40	n.s.	0.59	*	-0.07	n.s.	0.79	***						
woody	0.03	n.s.	0.25	n.s.	-0.03	n.s.	0.50	n.s.	0.46	n.s.				
earthy	0.26	n.s.	0.42	n.s.	-0.60	**	-0.15	n.s.	-0.02	n.s.	0.03	n.s.		
others	0.75	***	-0.17	n.s.	-0.42	n.s.	-0.51	**	-0.51	**	-0.04	n.s.	0.23	n.s.

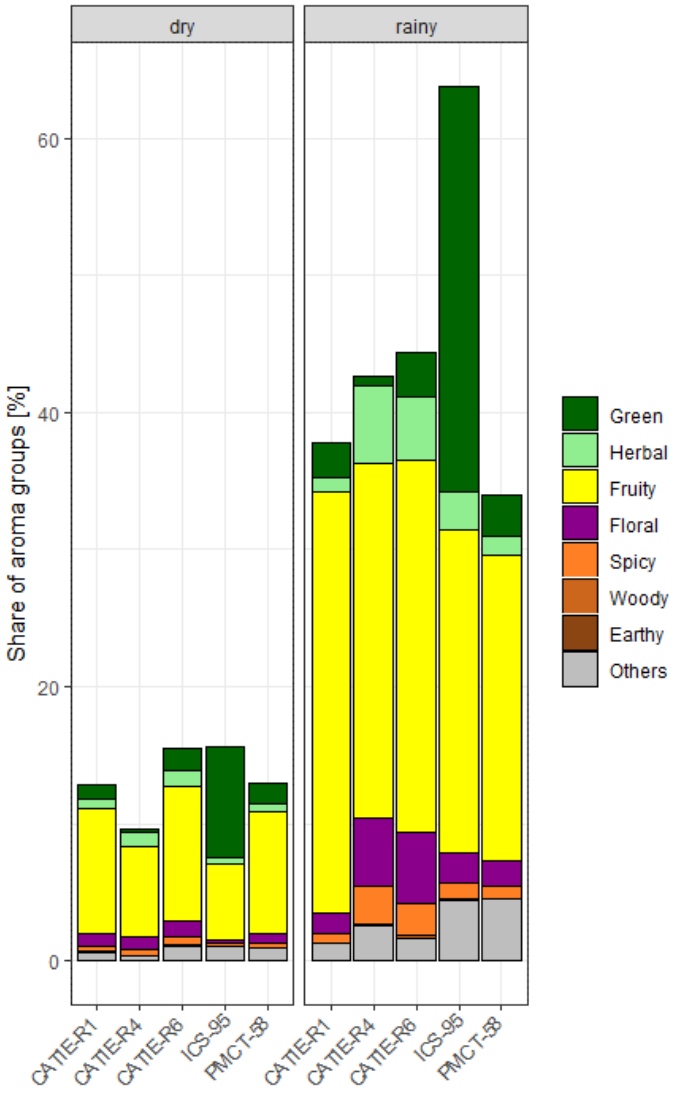

Fig. 4: Share of the aroma groups per varieties (pooled data from ripe and overripe fruits) displayed for two seasons (mean). Maximum total peak area was detected in one sample of the control genotype EET 62 and used as reference area (100 %).

Tab. 5: Results from linear mixed-effects models for the aroma groups and the factors variety (including the five varieties CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58), season (dry and rainy) and their interaction. F-value and level of significance: * p-value > 0.05; ** p-value > 0.01; *** p-value > 0.001; n.s. not significant.

	variety F-value		season F-value		variety:season F-value	
green	221.3	***	111.9	***	32.1	***
herbal	25.1	***	170.9	***	12.8	***
fruity	6.3	***	636.3	***	1.8	n.s.
floral	33.9	***	268.3	***	28.5	***
spicy	25.6	***	155.8	***	16.5	***
woody	15.4	***	0.2	n.s.	16.5	***
earthy	129.9	***	34.2	***	29.1	***
others	19.5	***	151.0	***	13.0	***

notes. However, cocoa producers often harvest fruits of different ripening stages, especially when harvest is not done regularly: some fruits are still unripe while others are almost overripe, which affects the aroma potential of the resulting bean composition. Otherwise, selecting or blending fruits based on their ripening stage may open up new avenues to obtain chocolate products with differentiated aromas and flavours.

The change in aroma properties of the fruit pulp during ripening implies a biological function. The predominantly lipophilic and volatile fine aroma compounds diffuse with increasing vapour pressure through the pericarp (personal communication LIEBEREI, 2015). This explains the observations and assumptions of WARREN and EMAMDIE (1992) that squirrels select fruits by odour and fruit colour and prefer certain cacao genotypes, such as SCA-6. In addition, they prefer ripe fruits over unripe fruits (WARREN and EMAMDIE, 1992). By selecting especially mature seeds from ripe fruits, rodents play a key role in the natural seed dispersal of the cacao tree.

Aroma intensification during the rainy season

The prevailing climate during fruit ripening strongly influences the aroma of the cacao pulp. In our study, the highest intensity of fine aromas was found in the samples of fruits harvested during the rainy season while highest aroma diversity was detected in fruits harvested during the dry season. It is well known that spicy and medicinal plants exposed to drought stress react with an increase in aromatic compounds deriving from their secondary metabolism (AL-GABBIESH et al., 2014; NOWAK et al., 2010). Under water deficit, the stomatal conductance of the cacao leaves decreases (LAHIVE et al., 2018), limiting photosynthesis and the protein metabolism (LARCHER, 1994). When carbon assimilation is reduced, the plant uses the available energy primarily to form lipids and structural elements (phospholipids in membranes) (TAIZ and ZEIGER, 2007). The down-regulation of the protein metabolism may reduce the synthesis of volatile fine aroma compounds from amino acids, which could explain the lower intensity of aroma components in the cacao fruit pulp in the dry season. In addition, when leaves heat up due to high ambient temperatures and radiation, the transpiration rate increases (LARCHER, 1994) and water may be removed from developed fruits. The increasing water withdrawal from the cacao fruits may have contributed to the observed changes in the synthesis of aroma compounds, as it is already described for other aromatic plants (AL-GABBIESH et al., 2014; NOWAK et al., 2010). In the rainy season, however, clouds and precipitation provide optimal growing conditions for the cacao tree, meaning sufficient energy is available for the synthesis of aroma-relevant pulp compounds and in high amounts. For several cocoa producing countries, changes in mean ambient temperature and precipitation patterns are expected or already observed (LÄDERACH et al., 2013, NIETHER et al., 2018,). The effect of seasons on the cacao fruit pulp aroma might be enhanced by a climate change induced extension of dry seasons, reducing the aroma intensity of the whole year harvest when blended up. That makes it even more important for the fine or flavour cocoa industry to recommend and establish cocoa production systems that are resilient to climate change like agroforestry systems (NIETHER et al., 2018) or support breeding for drought tolerant varieties (LAHIVE et al., 2018).

Conclusion

Previous findings that aroma intensity and aroma composition of fine or flavour cocoa varieties vary between genotypes were confirmed for five cacao selections from Costa Rica (CATIE-R1, CATIE-R4, CATIE-R6, ICS-95, PMCT-58). In addition, we showed for the first time analytically that aroma intensity and composition of the fruit pulp vary between different harvest seasons and change with the ripening stage of the fruit.

The results of the present study confirm that the fine aroma potential of the cacao pulp results from a complex combination of genetic pre-

disposition, as shown by the different varieties, environmental influences such as climatic conditions and the ripening stage of the fruit. However, the extent to which aroma compounds appear in the beans highly depends on the degree to which they can migrate from the pulp into the seeds during fermentation. Thus, the implementation of best practices during post-harvest management is another crucial step to maintain and increase aroma properties. Combining different cacao varieties may increase the aroma character of the chocolate, but only if ripe fruits are harvested. Industry and consumers should be aware that cocoa is a natural product and that the aroma composition of e.g. single origin chocolates made out of certain cacao varieties can vary throughout the year. Further research on the biochemical background is needed to understand the detailed mechanisms and transfer processes in the formation of fine aromas in *Theobroma cacao* L. This includes the role of bacteria and yeasts (naturally occurring or added starter cultures) in the migration of aroma compounds from the fruit pulp into the cotyledons during fermentation. Along with that, the variability and quantity of aroma relevant compounds within the existing varieties need to be examined, especially those compounds discriminating between fine or flavour cocoas and bulk cocoas.

Acknowledgements

This publication is based on a data set raised in a PhD thesis conducted under the guidance of Professor Reinhard Lieberei. It was supported by his comprehensive expertise on plant physiology and ability to capture interrelations between the cacao plant and post-harvest processing of cacao, leading our discussions beyond a typical biologist's horizon.

We thank Adriana Arciniegas and the field workers of the Cacao Breeding Program at CATIE for providing excellent cacao fruit material from IC3, Costa Rica. We also thank Sascha Rohn and Katrin Ulbrich (Food Chemistry, University of Hamburg, Germany) for providing the Headspace-GCMS-SPME equipment and for great assistance in fruit pulp analytics. This research was partly supported by the Chocolate Manufacturer Rausch GmbH, Germany.

Conflict of Interest

No potential conflict of interest was reported by the authors.

References

Al-Gabbiesh, A., Kleinwächter, M., Selmar, D., 2014: Influencing the Contents of Secondary Metabolites in Spice and Medicinal Plants by Deliberately Applying Drought Stress during their Cultivation. Jordan J. Biol. Sci. 8(1), 1-10.

Andersson, M., Koch, G., Lieberei, R., 2006: Structure and function of the seed coat of *Theobroma cacao* L. and its possible impact on flavour precursor development during fermentation. J. Appl. Bot. Food Qual. 80, 48-62.

Aprotosoaie, A.C., Vlad Luca, S., Miron, A., 2016: Flavor Chemistry of Cocoa and Cocoa Products – An Overview. Compr. Rev. Food Sci. F., Vol. 15. DOI: 10.1111/1541-4337.12180

Belitz, H.-D., Grosch, W., Schieberle, P., 2009: Food Chemistry. 4th Edition, Springer-Verlag.

Chetschik, I., Kneubühl, M., Chatelain, K., Schlüter, A., Bernath, K., Hühn, T., 2017: Investigations on the Aroma of Cocoa Pulp (*Theobroma cacao* L.) and Its Influence on the Odor of Fermented Cocoa Beans. J. Agricult. Food Chem. 66(10), 2467-2472. DOI: 10.1021/acs.jafc.6b05008

De Araujo, Q.R., Baligar, V.C., De A. Loureiro, G.A.H., de Souza Júnior, J.O., Comerford, N.B., 2017: Impact of soils and cropping systems on mineral composition of dry cacao beans. J. Soil Sci. Plant Nut. 17(2), 410-428. DOI: 10.4067/S0718-95162017005000030

Deluc, L.G., Quilici, D.R., Decendit, A., Grimplet, J., Wheatley, M.D., Schlauch, K.A., Mérillon, J.M., Cushman, J.C., Cramer, G.R., 2009: Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. BMC Genomics 10(1), 212. DOI: 10.1186/1471-2164-10-212

Delgado, R.A., Solís, Z.K., Suárez-Capello, C., Peres Gramacho, K., Gelape Faleiro, F., Vanderlei Lopes, U., 2003: Genetic diversity among cocoa (*Theobroma cacao* L.) Elite Clones from Ecuador. 14th International Cocoa Research Conference, Vol. 1, 13-17 October 2003, Ghana.

Eskes, A., Guarda, D., Garcia, L., Garcia, R., 2007: Is genetic variation for sensory traits of cocoa pulp related to fine-flavour cocoa traits? Ingenic Newsletter (11), 22-28.

Horikoshi, M., Tang, Y., 2016: ggfortify: Data Visualization Tools for Statistical Analysis Results. https://CRAN.R-project.org/package=ggfortify

Hui, Y., 2010: Handbook of Fruit and Vegetable Flavors. John Wiley & Sons, 407.

Kadow, D., Bohlmann, J., Phillips, W., Lieberei, R., 2013: Identification of main flavour components in two genotypes of the cocoa tree (*Theobroma cacao* L.). J. Appl. Bot. Food Qual. 86, 90-98. DOI: 10.5073/JABFQ.2013.086.013

Kuznetsova, A., Brockhoff, P.B., Christensen, R.H.B, 2017: lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82(13), 1-26. DOI: 10.18637/jss.v082.i13

Lahive, F., Hadley, P., Daymond, A.J., 2018: The physiological responses of cacao to the environment and the implications for climate change resilience. A review. Agron. Sustain. Dev. (2019) Vol. 39(5). DOI: 10.1007/s13593-018-0552-0

Lan-Phi, N.T., Shimamura, T., Ukeda, H., Masayoshi, S., 2009: Chemical and Aroma Profiles of Yuzu (*Citrus junos*) Peel Oils of Different Cultivars. Food Chem. 115(3). DOI: 10.1016/j.foodchem.2008.12.024

Läderach, P., Martinez-Valle, A., Schroth, G., Castro, N., 2013: Predicting the future climatic suitability for cocoa farming of the world's leading producer countries, Ghana and Côte d'Ivoire. Climatic Change, Vol. 119, 841-845. DOI: 10.1007/s10584-013-0774-8

Larcher, W., 1994: Ökophysiologie der Pflanzen: Leben, Leistung und Streßbewältigung der Pflanzen in ihrer Umwelt. 5. Auflage. UTB für Wissenschaft. Verlag Eugen Ulmer Stuttgart. ISBN 3-8252-8074-8

Leal, A., 2005: Caracterización de árboles superiores de cacao (*Theobroma cacao* L.) seleccionados por el programa de mejoramiento genético del CATIE. Thesis. http://hdl.handle.net/11554/4571

Lenth, Russel, V., 2016: Least-Squares Means: The R Package lsmeans. J. Stat. Softw. 69(1), 1-33. DOI:10.18637/jss.v069.i01

Lieberei, R., Reisdorff, C., 2012: Nutzpflanzen. Begründet von Franke, W. (1976). 8th Edition, Georg Thieme Verlag KG, Stuttgart. ISBN: 978-3-13-530408-3

Lockwood, G., Eskes, A.B., 1996: Relationship between cocoa variety and quality. In: Cocoa Meetings – The various aspects of quality. Seminar Proceedings, 159-167. Montpellier, France, 1995. CIRAD, France.

Mata-Quirós, A., Arciniegas-Leal, A., Phillips-Mora, W., Meinhart, L., Zhang, D., 2017: Understanding the genetic structure and parentage of the clonal series of cacao UF, CC, PMCT AND ARF preserved in the International Cacao Collection at CATIE (IC3). International Symposium on Cocoa Research. Lima, Perú. 13-17 November 2017.

Mosciano, G., 1989: Perfumer & Flavorist Magazine 14, No. 6, 47.

Mosciano, G., 1990a: Perfumer & Flavorist Magazine 15, No. 2, 69.

Mosciano, G., 1990b: Perfumer & Flavorist Magazine 15, No. 4, 59.

Mosciano, G., 1991a: Perfumer & Flavorist Magazine 16, No. 2, 49.

Mosciano, G., 1991b: Perfumer & Flavorist Magazine 16, No. 3, 79.

Mosciano, G., 1991c: Perfumer & Flavorist Magazine 16, No. 4, 45.

Mosciano, G., 1991d: Perfumer & Flavorist Magazine 16, No. 5, 71.

Mosciano, G., 1992a: Perfumer & Flavorist Magazine 17, No. 4, 33.

Mosciano, G., 1992b: Perfumer & Flavorist Magazine 17, No. 5, 127.
Mosciano, G., 1993a: Perfumer & Flavorist Magazine 18, No. 2, 38.
Mosciano, G., 1993b: Perfumer & Flavorist Magazine 18, No. 4, 51.
Mosciano, G., 1995a: Perfumer & Flavorist Magazine 20, No. 1, 31.
Mosciano, G., 1995b: Perfumer & Flavorist Magazine 20, No. 4, 23.
Mosciano, G., 1995c: Perfumer & Flavorist Magazine 20, No. 5, 49.
Mosciano, G., 1996a: Perfumer & Flavorist Magazine 21, No. 1, 33.
Mosciano, G., 1996b: Perfumer & Flavorist Magazine 21, No. 5, 49.
Mosciano, G., 1997a: Perfumer & Flavorist Magazine 22, No. 2, 69.
Mosciano, G., 1997b: Perfumer & Flavorist Magazine 22, No. 6, 41.
Mosciano, G., 1998: Perfumer & Flavorist Magazine 23, No. 5, 49.
Mosciano, G., 2000: Perfumer & Flavorist Magazine 25, No. 6, 26.
Mosciano, G., 2001a: Perfumer & Flavorist Magazine 26, No. 2, 40.
Mosciano, G., 2001b: Perfumer & Flavorist Magazine 26, No. 3, 80.
Mosciano, G., 2009: Perfumer & Flavorist Magazine 6, 52.
Motamayor, J.C., Lachenaud, P., Wallace da Silva de Mota, J., Loor, R., Kuhn, D.N., Brown, J.S., Schnell, R.J., 2008: Geographic and Genetic Population Differentiation of the Amazonian Chocolate Tree (*Theobroma cacao* L.). PLoS ONE, Vol. 3(10). e3311. DOI: 10.1371/journal.pone.0003311
Niether, W., Smit, I., Armengot, L., Schneider M., Gerold, G., Pawelzik, E., 2017: Environmental Growing Conditions in Five Production Systems Induce Stress Response and Affect Chemical Composition of Cocoa (*Theobroma cacao* L.) Beans. J. Agricult. Food Chem. 65(47), 10165-10173. DOI: 10.1021/acs.jafc.7b04490
Niether, W., Armengot, L., Andres, C., Schneider, M., Gerold, G., 2018: Shade trees and tree pruning alter throughfall and microclimate in cocoa (*Theobroma cacao* L.) production systems. Ann. For. Sci. 75(38). DOI: 10.1007/s13595-018-0723-9
Nowak, M., Kleinwächter, M., Manderscheid, R., Weigel, H.-J., Selmar, D., 2010: Drought stress increases the accumulation of monoterpene in sage (*Salvia officinalis*), an effect that is compensated by elevated carbon dioxide concentration. J. Appl. Bot. Food Qual. 83, 133-136.
Nozaki, M., Suzuki, N., Oshikubo, S., 1997: Production of Chiral Flavour components by enzymic methods. In: Taylor, A.J., Mottram, D.S. (eds.), Flavour Science: Recent Developments, 168-171. Elsevier. ISBN: 978-1-85573-779-2
Phillips, W., Castillo, J., Arciniegas, A., Mata, A., Sánchez, A., Leandro, M., Astorga, C., Motamayor, J., Guyton, G., Seguine, E., Schnell, R., 2009: Overcoming the Main Limiting Factors of Cocoa Production in Central America through the Use of Improved Clones Developed at CATIE. Proceedings of the 16th International Cocoa Research Conference, Bali, Indonesia, 93-99. ISBN: 978-065-959-5
Phillips-Mora, W., Arciniegas-Leal, A., Mata-Quirós, A., Motamayor-Arias, J.C., 2013: Catalogue of cacao clones selected by CATIE for commercial plantings. Technical series. Technical manual / CATIE; Vol. 105, 68. ISBN: 978-9977-57-590-2
Phillips-Mora, W., Mata-Quirós, A., Arciniegas-Leal, A., 2017: Generation of Cacao Clones with durable resistant against frost pod rot (*Moniliophthora roreri* (Cif. & Par.) Evans et al.). International Symposium on Cocoa Research. Lima, Perú. 13-17 November 2017.
Pino, J.A., Ceballos, L., Quijano, C., 2010: Headspace Volatiles of *Theobroma cacao* L. Pulp From Colombia. J. Essent. Oil Res. 22, Issue 2, 113-115. DOI: 10.1080/10412905.2010.9700276
Pires, J.L., Mattos Cascardo, J.C., Lambert, S.V., Figuiera, A., 1998: Increasing cocoa butter yield through genetic improvement of *Theobroma cacao* L.: Seed fat variability, inheritance and association with seed yield. Euphytica 103, 115-121. Kluwer Academic Publishers
R Core Team, 2019: R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Rodriguez-Campos, J., Escalona-Buendía, H.B., Contreras-Ramos, S.M., Orozco-Avila, I., Jaramilloflores, E., Lugo-Cervantes, E., 2011: Effect of fermentation time and drying temperature on volatile compounds in cocoa. Food Chem. 132(1), 277-288. DOI: 10.1016/j.foodchem.2011.10.078
Rohsius, C., 2007: Die Heterogenität der biologischen Ressource Rohkakao (*Theobroma cacao* L.). Dissertation. Department of Biology, University of Hamburg. URL: http://ediss.sub.uni-hamburg.de/volltexte/2008/3817/pdf/C_Rohsius_Dissertation.pdf
Rohsius, C., Elwers, S., Lieberei, R., 2010: Cocoa Atlas. Hamburg/Bonn: Deutsche Stiftung der Kakao- und Schokoladenindustrie. DVD Edition 2010, ISBN: 9-783980-886666
Savoi, S., Wong, D.C.J., Arapitsas, P., Miculan, M., Bucchetti, B., Peterlunger, E., Fait, A., Mattivi, F., Castellarin, S.D., 2016: Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.) BMC Plant Biol. 16, 67. DOI: 10.1186/s12870-016-0760-1
Savoi, S., Herrera, J.C., Carlin, S., Peterlunger, E., 2020: From grape berries to wines: drought impacts on key secondary metabolites. OENO One 54(3), 569-582. DOI: 10.20870/oeno-one.2020.54.3.3093
Schieberle, P., Pfnuer, P., 1999: Characterization of key odorants in chocolate. In: Teranishi, R. et al. (eds.), Flavor Chemistry: 30 Years of Progress, 147-153. Kluwer Academic/Plenum, New York.
Schwan, R.F., Wheals, A.W., 2004: The microbiology of cocoa fermentation and its role in chocolate quality. Crit. Rev. Food Sci. Nutr. 44, 205-221.
Suburg, H., Panten, J., 2006: Common Fragrance and Flavor Materials. 5th Edition. WILEY-VCH Verlag GmbH& Co. KGaA, Weinheim. ISBN: 3-527-31315-X
Sukha, D., 2008: The influence of processing location, growing environment and pollen donor effects on the flavour and quality of selected cacao (*Theobroma cacao* L.) genotypes. Thesis, University of the West Indies (Saint Augustine, Trinidad and Tobago). URL: http://hdl.handle.net/2139/4855
Taiz, L., Zeiger, E., 2007: Plant Physiology. 4. Edition, Spektrum Akademischer Verlag, Springer. ISBN: 978-3-8274-1865-4
Voigt, J., Lieberei, R., 2014: Biochemistry of Cocoa Fermentation. In: Schwan, R.F., Fleet, G.H. (eds.), Cocoa and Coffee Fermentations, 185-217. CRC Press, Taylor & Francis Group. ISBN: 9781439847916
Warren, J., Emamdie, D., 1992: Rodent resistance in cacao, *Theobroma cacao* L.. Tropical Agriculture (Trinidad) 70(3), 286-288.
Wickham, H., Navarro, D., Lin Pedersen, T., 2009: ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
Wickham, H., 2011: The Split-Apply-Combine Strategy for Data Analysis. Journal of Statistical Software 40(1), 1-29. URL: http://www.jstatsoft.org/v40/i01/
Ziegleder, G., 1990a: Linalool contents as characteristic of some flavour grade cocoas. Zeitschrift für Lebensmittel-Untersuchung und Forschung 191, 306-309. Springer Verlag.

ORCID
Elsa Hegmann 0000-0001-8940-0243
Wiebke Niether 0000-0002-7776-1268
Wilbert Phillips 0000-0002-7707-3705
Christina Rohsius 0000-0002-5858-717X

Address of the corresponding author:
Elsa Hegmann, E-mail: ehegmann@rausch.de

Supplementary material

Tab. 1A: Amount of cacao fruit pulp samples per variety, season and ripening stage. Studied ripening stages: 1 = unripe; 2= ripe; 3= overripe

Variety	Season	Ripening stage	Amount fruits	Samples
PMCT-58	dry	1	1	2
PMCT-58	dry	2	3	6
PMCT-58	dry	3	2	4
PMCT-58	rainy	2	3	9
ICS-95 (T1)	dry	1	2	4
ICS-95 (T1)	dry	2	2	4
ICS-95 (T1)	dry	3	2	4
ICS-95 (T1)	rainy	1	1	3
ICS-95 (T1)	rainy	2	2	6
CATIE-R1	dry	1	3	6
CATIE-R1	dry	2	2	4
CATIE-R1	dry	3	1	2
CATIE-R1	rainy	3	3	9
CATIE-R4	dry	1	2	4
CATIE-R4	dry	2	2	4
CATIE-R4	dry	3	2	4
CATIE-R4	rainy	3	3	9
CATIE-R6	dry	1	2	4
CATIE-R6	dry	2	2	4
CATIE-R6	dry	3	2	4
CATIE-R6	rainy	3	2	6
SCA-6	rainy	2	2	3
SCA-6	dry	2	2	3
EET 62	rainy	2	2	6
EET 62	dry	2	2	4