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# New Resistant Cocoa Selections from Costa Rica have Fine Aroma Potential

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## 1. Abstract

In this research the aroma potential of six new cocoa genotypes (CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58, ICS-95 (T1) and CC-137) selected at CATIE, Costa Rica, was studied. Since 2007, these high-yielding and disease resistant cocoa clones are cultivated by cocoa farmers and cooperatives throughout Central-America, however, detailed information on the individual aroma potential is lacking. The main factors which interact and define the aroma characteristics of the traded product "raw cocoa" are genotype, post-harvest management, cultivation site and climatic conditions. Cocoa liquors produced from "Fine or Flavour Cocoa" are characterized by chocolate aroma and additional fine aroma notes described as e.g. fruity, floral or nutty. These aroma compounds originate from plant secondary metabolites and their derivatives, such as terpenes, alcohols, esters, aldehydes, methyl ketones, with most of them being formed or stored in the fruit pulp. Apparently, these aromas migrate into the seed during fermentation and contribute to the organoleptic character of the cocoa bean. The aim of this study was to determine whether the new cocoa selections from CATIE have a "Fine or Flavour"- potential and which post-harvest treatment can be advised to exploit the full aroma potential of these clones. Monoclonal fermentations and fruit pulp analytics were carried out in Costa Rica and at the University of Hamburg, resp., in order to study and identify the main components involved in aroma formation of these new resistant varieties. The results demonstrate that fermentation procedure and -duration have to be adjusted to the clonal material and environmental conditions, because both highly influence the fermentation processes. The analyzed clonal fruit pulps of the CATIE-Selections displayed a basic content of the two dominating esters 2-pentanol acetate and 2-heptanol acetate, combined with a distinctive mixture of various minor compounds which form the genotype-specific fine aroma. Thus, the individual aroma bouquet is defined by rather minor compounds of lower concentrations. Furthermore, the results show that fruit pulp's aroma composition and aroma intensity vary with the prevailing climate in the phase of cocoa pod ripening, as well as the ripening status of the pods.

## 2. Introduction

Trade and industry differentiate between bulk cocoa and fine or flavour cocoa. All genotypes of *Theobroma cacao* L. have the ability to develop chocolate aroma during roasting as it derives from endogenous compounds stored in the cotyledons (Ziegleder, 1994; Thompson et al., 2001). Fine aroma potential, however, is defined by the given cocoa genotype. Fine cocoas are characterized by the "normal" chocolate aroma, but additionally have special aroma components based on secondary plant metabolites such as terpenes, alcohols, esters, aldehydes or methyl ketones. From previous studies it is known that these volatile and non-volatile aroma components are stored predominantly in the fruit pulp surrounding the cocoa seeds (Ziegleder, 1990a, Eskes et al., 2007, 2009). Apparently, they are relocated into the cotyledons during fruit ripening or fermentation, resp. (Eskes et al., 2009, Kadow et al. 2013).
According to ICCO (2015) and Faostat (2017), the share of fine or flavour cocoa in the global raw cocoa production decreased from 40-50 % to 5-8 % since the beginning of the 20th century due to an increase in bulk cocoa production. However, in recent years the global cocoa trade is characterized by a growing demand for these fine or flavour cocoas (ICCO, 2015, Eskes et al., 2007). Farmers growing fine or flavour cocoas receive a premium per ton of raw cocoa, which varies according to supply and demand for the respective region and the cultivated cocoa varieties (ICCO, 2015). At present, the vast majority of cocoas grown belong to the Forastero types (bulk cocoa). These cocoas show high disease tolerance, enhanced productivity and have a pronounced chocolate flavour while lacking fine aroma notes. In contrast, Criollo cocoas such as "Porcelana" and "Pentagona" from Venezuela are characterized by fine aroma notes and the

cocoa fruits contain white/pale seeds (no anthocyanidines) with high caffeine content (Rohsius, 2007). Due to low productivity and high susceptibility to diseases and pathogen attack, Criollo cocoas are barely existent today. By means of molecular markers Motamayor et al. (2008) were able to show how natural hybridization has led to a great mixture of genetic traits, thus, classification of cocoa types according to their geographical origin is no longer viable from a scientific point of view. However, traders continue to divide raw cocoa into four groups according to the origin: Criollo, Forastero, Trinitario and Arriba-Nacional (a genotype population grown Ecuador characterized by unique floral aroma notes) (ICCO, 2015). Today, the most common fine cocoa is a hybrid of Criollo and Forastero, the so called Trinitario (Afoakwa et al., 2008). Cotyledons may appear white, pink and purple and present fine aromas, especially fruity-floral notes (Rohsius, 2007).

Since the 1960s, most of the Trinitario varieties grown in cocoa plantations in Central America were directly planted as seedlings and quickly lost their vigor and resistance to devastating diseases, in particular "frosty pod" (*Moniliophthora roreri*) (Somarriba, 2013). The impact of "frosty pod" endangers the existence of thousands of cocoa plants and by this the income of many smallholder families (overview articles Phillips-Mora and Wilkinson, 2007). In Costa Rica, "frosty pod" hit the cocoa culture in 1978 at times of highest production (10k MT). Within two years, the country's annual cocoa production declined by half and reached its lowest level with 343 MT in 2005 (Faostat, 2015). Different approaches were implemented in order to combat the severe situation. Within the scope of the Cocoa Genetic Improvement Program at the Tropical Agricultural Research and Higher Education Centre (CATIE) located in Turrialba, Costa Rica, a number of improved clones were developed (Phillips et al., 2009). Over 11 years of data collection and selection pressure on important field quality parameters let to the release of 6 new cocoa varieties in 2007: CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58, ICS-95 (T1)[1] and CC-137 (Phillips et al., 2013). These cocoa clones are characterized by enhanced yield potential and high tolerance to the fungal diseases "frosty pod" (*Moniliophthora rorei*) and "black pod" (especially *Phytophthora palmivora*), resp. Today, they are grown in all Central American countries, Mexico and Brazil (Karp, 2017). However, information about the aroma quality of these cocoa selections was lacking.

Therefore, the aim of this research carried out between 2012-2015 (Hegmann, 2015), was to determine whether

a) the new cocoa clones from CATIE can be classified as fine or flavour cocoas (fruit pulp analytics),
b) biochemical and biophysical sequences in course of fermentation vary with the clonal material (monoclonal fermentations) and
c) whether a specific post-harvest treatment can be advised in order to exploit their full potential for high raw cocoa quality (monoclonal fermentations).

This paper summarizes the main results obtained from fresh fruit pulp analytics of CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58 and ICS-95 (T1).

## 3. Materials and Methods

### 3.1. Genetic material

New cocoa selections from CATIE, Costa Rica: CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58 and ICS-95 (T1). Control genotypes with high amounts of fine aroma volatiles: EET 62 ("Nacional" cocoa, according to Delgado et al., 2003), SCA-6 ("Contamana" cocoa, according to Motamayor et al., 2008).

Data shown in Tab.1 derive from the latest version of the CATIE clone catalogue published by Phillips et al. in 2013.

**Tab 1.: Genetic background of the studied clones from CATIE**

| Variety | Pedigree |
|---|---|
| CATIE-R1 | UF-273 T1 X CATIE-1000[2] |
| CATIE-R4 | UF-273 T1 X PA-169 |
| CATIE-R6 | UF-273 T1 X PA-169 |
| ICS-95 (T1) | Unknown Trinitario x Criollo hybrid |

[1] ICS-95 is a cocoa clone from Trinidad (Imperial College Selections). T1 stands for Type 1. ICS-95 (T1) does not differ from the original genotype on molecular level, but was improved in various selection studies at CATIE.

[2] CATIE-1000 was selected in the 1970s from the cross Pound-12 x Catongo for its good production and tolerance to black pod (Phillips et al., 2013).

| | |
|---|---|
| PMCT-58[3] | Trinitario hybrid of unknown parents |

3.2. Analyses of volatile aroma compounds in fresh cocoa fruit pulps

Cocoa fruits of the different clones were provided by CATIE, Costa Rica, during rainy season (November 2012) and dry season (April 2013), resp.. Fruits were harvested in the morning hours at the International Cocoa Collection (IC3) and sent to the University of Hamburg the same day by express (3-4 days). In the dry season unripe, ripe and overripe fruits, resp., were sent in order to study the potential influence of the ripening stage on the final pulp aroma composition and intensity.
Cocoa fruits were sliced longitudinally, fruit pulp and testa removed from the cotyledons with a scalpel (5 g of each) and directly weighed into a 20mL Headspace Crimp Neck Vial N 20 (Company Macherey Nagel). Vials were closed gastight using an aluminum crimp cap type N20 (8 mm, Septum: blue silicone / PTFE colorless, 3 mm, company Macherey Nagel). Samples were stored at -80 ° C until quantitative determination of the sample components by gas chromatography-mass spectrometry (GCMS) (Tab. 2a). For analyses, 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 fiber (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.2b). 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).

**Tab 2.: GCMS structure and conditions**

a) Gas chromatograph 6890plus

| | | | | |
|---|---|---|---|---|
| Injector | KAS 4 | | | |
| Program | Start-Temp.: 200°C, hold for 30 s;<br>12 °C min$^{-1}$ at 240°C, hold for 10 min | | | |
| Front Inlet | Mode: pulsed splitless<br>pressure: 48,5 kPa<br>pulse pressure: 250 kPa<br>pulse time: 30 s<br>purge flow: 40,1 mL min$^{-1}$<br>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$^{-1}$]** | **Temp. [°C]** | **Hold [min]** |
| | Initial | - | 40 | 3 |
| | Ramp 1 | 3 | 100 | 0 |
| | Ramp 2 | 10 | 150 | 0 |
| | Ramp 3 | 15 | 240 | 5 |

b) Mass-spectrometer S 5973

| | |
|---|---|
| Transferline | 300 °C |
| MS Source | 230 °C |
| MS Quad | 150 °C |
| Mass scan | 40-400 |
| Solvent Delay | 0,5 min |

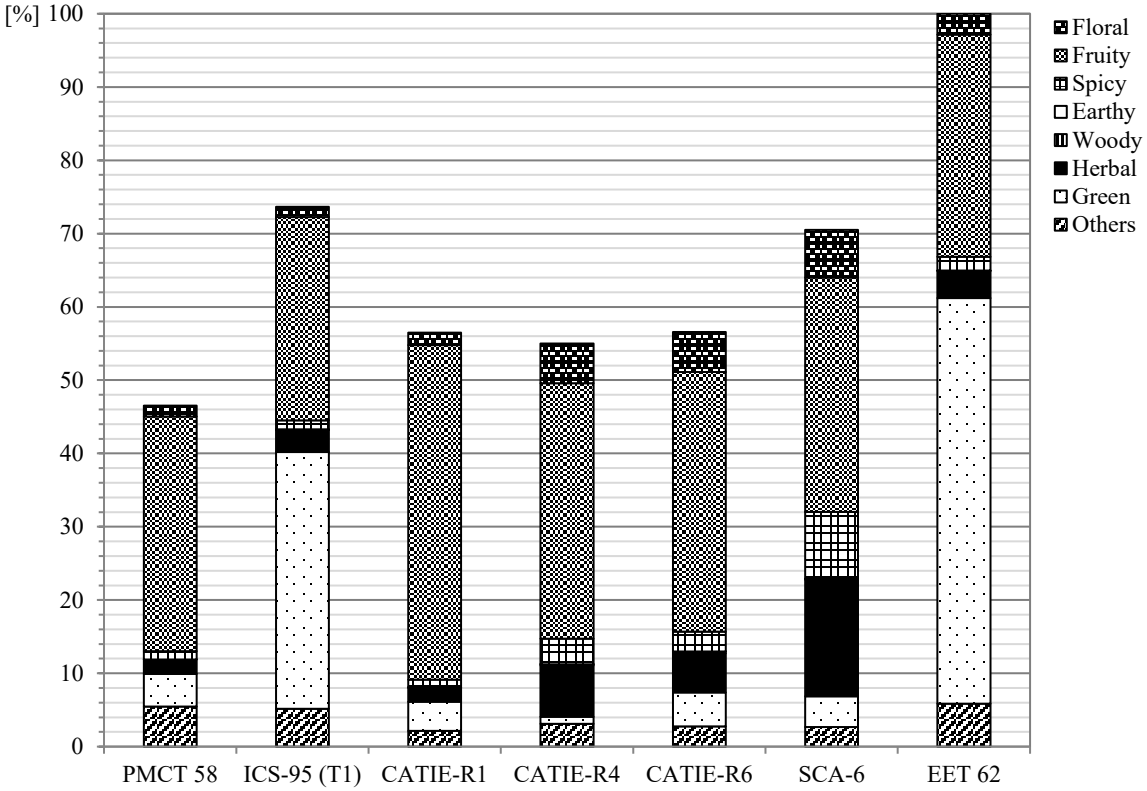
[3] The clone PMCT-58 is a selection from earlier breeding trials (PHILLIPS et al., 2009). According to the database of the University of Reading PMCT-58 was first documented in 1991 (Reading, 2015), with PMCT for *Programa Mejoramiento de Cultivos Tropicales*.

## 4. Results and Discussion

A total of 57 volatile aroma components from the group of aromatics, terpenes, esters, aldehydes and ketones were identified in the fruit pulps of the cocoa clones PMCT-58, ICS-95 (T1), CATIE-R4, CATIE-R1, CATIE-R6 and the control genotypes SCA-6 and EET 62 (Tab. 3, Tab.4). However, some of them could not be clearly assigned to a specific aroma and are not listed here. All results are based on fresh weight per sample (5 g). Odour descriptions mentioned in Fig.1 and Tab.3 to Tab.6 derive from 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*) as well as Surburg and Panten (2006), Nozaki et al. (1997), Hui (2010), Lan-Phi et al. (2009). The aim of the present research was not a precise quantification of aroma compounds, but rather to study and identify the main factors involved in aroma formation in fruit pulps of *Theobroma cacao* L..

A multi-factorial ANOVA followed by a post-hoc Tukey HSD test for unequal N, with $\alpha = 0.05$, showed a statistically significant influence of the genotype and the season on the proportion of the individual fine aroma components in the total pulp aroma. The control genotype EET-62 indicated the highest amount of volatile aromas in its pulp of mature fruits, with a mean total peak area of 5.29E + 09. It was used as reference area for evaluation of the other genotypes. ICS-95 (T1) showed a mean total peak area of 3.89E + 09 (equal to 73,6 % of EET 62), followed by SCA-6 with 3.73E + 09 (equal to 70,5 % of EET 62). CATIE-R6, CATIE-R1 and CATIE-R4 indicated similar average total peak areas with 2.99E + 09 (56,6 %), 2.99E + 09 (56,5 %) and 2.91E + 09 (55,0 %), resp. (Fig.1).
The genotype PMCT-58 contained the lowest amounts of volatile aromas. The size of its average total peak area (2,46E+09) was less than half of EET 62 (46,5 %) (Fig.1).
For all clones except EET 62 and ICS-95 (T1) the pulp-aroma was dominated by fruity notes (Fig.1). In EET 62 and ICS-95 (T1) green-herbal aromas prevailed with 2-heptanol acetate being the major component (Tab.3). All other clones contained significantly lower amounts of this ester.
Characteristic for the pulp-aroma of SCA-6 were terpenes and alcohols with spicy and floral odors, similar to the genotypes CATIE-R4 and CATIE-R6 (Tab.4). Compared to the other genotypes, CATIE-R4 and CATIE-R6 showed highest amounts of the acyclic monoterpenes myrcene, trans-ocimene and alpha-ocimene as well as the floral terpene-alcohol linalool. In addition, traces of the sesquiterpenes alpha-bergamotene, alpha-copaene (both woody-spicy aromas), trans-beta-farnesene (fruity) and the ester linalyl acetate (vegetable nuance) were detected in their fruit pulps (Tab.4). The similarities between CATIE-R4 and CATIE-R6 might be explained by their genetic background, because both clones have the same parental genotypes (UF 273 (T1) x PA-169). The analyzed fruit pulps of CATIE-R1 primarily consisted of alcohols, esters and ketones, with large amounts of different fruity aromas (Fig.1). These volatiles accounted for more than 80% of all identified pulpa aromas in this genotype, with 2-pentanol acetate and 2-pentanone being the most dominating ones. Among others, cis-linalooloxide, trans-linalooloxide and epoxylinalool (pyranoid) add floral and earthy notes (Suburg and Panten, 2006) to the fruit pulp aroma of CATIE-R1. Monoterpenes play a minor role in the aroma formation of this clone. However, CATIE-R1 showed many traces of complex sesquiterpenes, similar to CATIE-R4 and CATIE-R6. This ability might be related to their identical maternal parent (UF-273 (T1), because fruit pulp properties derive from tissues given by the mother plant (mesocarp and endocarp) (Andersson et al., 2006).
We observed various similarities between the aroma characters of EET 62 and ICS-95 (T1), resp. In both varieties, secondary alcohols, esters and methyl ketones play a decisive role in the aroma formation. Kadow et al. (2013) mentioned 2-heptanol acetate, 2-heptanol, 2-heptanone and 2-nonanone as key components in the aroma of EET 62. This agrees with the results of the present study where additionally, 2-octanol acetate and 2-nonanol were detected in EET 62 and ICS-95 (T1) (Tab.4). Both volatile components add citrus notes to the aroma characters of the fruit pulps of these genotypes (Mosciano, 1990). Although PMCT-58 demonstrated the lowest aroma intensity of all genotypes it contained traces of different minor components, such as the methyl ketone acetophenone with organoleptic properties described as orange-blossom and sweet, as well as its derivatives 1-phenylethyl acetate (gardenol) and 1-phenylethanol, which are also classified as floral aromas (Suburg and Panten, 2006). We found, the prevailing climate during ripening affects the aroma of cocoa fruit pulps: highest amounts of fine aromas were found in the cocoa pulps of fruits harvested in the rainy season. However, in some CATIE-Clones the aroma-diversity was higher in the dry season (Tab.4 and 5). Spice and medicinal plants suffering drought stress react with an increase in the biosynthesis of aroma compounds (Al-Gabbiesh et al., 2014, Nowak et al. 2010). This raises the question which ecophysiological mechanisms affect pulp properties in *Theobroma cacao* L. In general, lack of water limits the protein metabolism (Larcher, 1994). It can be assumed that volatiles originating from amino acids are less synthesized during periods of water scarcity. Assimilation rate is limited, thus, the

cocoa plant uses the available energy primarily for the formation of storage lipids and structural elements (phospholipids in membranes) (Taiz and Zeiger, 2007).




| | PMCT 58 | ICS-95 (T1) | CATIE-R1 | CATIE-R4 | CATIE-R6 | SCA-6 | EET 62 |
|---|---|---|---|---|---|---|---|
| Floral | 1.50 ± 0.4 | 1.41 ± 0.4 | 1.74 ± 1.5 | 5.36 ± 0.4 | 5.45 ± 1.6 | 6.48 ± 2.0 | 2.85 ± 0.9 |
| Fruity | 31.99 ± 7.5 | 27.70 ± 5.0 | 45.64 ± 7.9 | 34.87 ± 11.2 | 35.43 ± 8.0 | 31.98 ± 1.3 | 30.31 ± 0.9 |
| Spicy | 1.14 ± 0.7 | 1.24 ± 0.7 | 1.04 ± 2.0 | 3.50 ± 0.2 | 2.72 ± 1.6 | 9.11 ± 1.4 | 1.87 ± 1.1 |
| Earthy | - | 0.07 | 0.01 | 0.01 | 0.02 | 0.01 | 0.04 |
| Woody | 0.01 | 0.01 | 0.07 | 0.17 | 0.24 | 0.15 | 0.01 |
| Herbal | 1.96 ± 0.5 | 3.01 ± 0.7 | 1.89 ± 1.8 | 6.97 ± 0.6 | 5.33 ± 1.5 | 15.90 ± 5.3 | 3.70 ± 1.2 |
| Green | 4.46 ± 1.3 | 35.06 ± 10.6 | 3.99 ± 0.3 | 0.97 ± 0.3 | 4.65 ± 1.4 | 4.19 ± 1.3 | 55.37 ± 16.7 |
| Others | 5.46 ± 0.7 | 5.14 ± 1.0 | 2.13 ± 0.6 | 3.12 ± 0.5 | 2.75 ± 0.8 | 2.68 ± 0.5 | 5.85 ± 1.5 |

**Fig.1: Aroma compositions of the fruit pulps of ripe and overripe cocoa fruits of the CATIE-selections PMCT-58, ICS-95 (T1), CATIE-R1, CATIE-R4, CATIE-R6 and the control genotypes SCA-6 and EET 62, resp.**

Given are the average amounts [%] of volatile aroma components with floral, fruity, spicy, earthy, woody, herbaceous, green organoleptic properties, averaged over both seasons (rainy season, dry season). The group „others" refers to those volatile components that could not be assigned to a specific aroma. The detected aroma compounds are clustered in aroma groups according to the suggestions of 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*) as well as Surburg and Panten (2006), Nozaki et al. (1997), Hui (2010), Lan-Phi et al. (2009). Maximum total peak area was detected in EET 62 and therefore used as reference area (100%). n per clone = 6-19. Error values: standard deviations. Amounts <0,1% are considered as traces.

**Tab.3: Major and minor volatile aroma components detected in fruit pulps of different cocoa varieties**

A: Major compounds with peak areas < 7,0E+09; B: Major compounds with peak areas < 1,0E+09; C: Minor compounds with peak areas < 5,0E+08

Odour descriptions mentioned in Fig.1 and Tab.3 to Tab.6 derive from 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*) as well as Surburg and Panten (2006), Nozaki et al. (1997), Hui (2010), Lan-Phi et al. (2009). ANOVA and *post-hoc* Tukey HSD test for unequal N, with $\alpha = 0.05$ and $p < 0.01$.

| Category | Aroma compound | CAS-No. | Group | Odor | Odor description | Identified in | | | | | | | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | PMCT 58 | ICS-95 (T1) | CATIE-R1 | CATIE-R4 | CATIE-R6 | SCA-6 | EET 62 | |
| A | 2-pentanol acetate | 626-38-0 | ester | fruity | tropical, Orange, musty, green, unripe, fruity, Banana, slight nutty nuance | x | x | **x** | **x** | **x** | x | x | all significantly higher in rainy season |
| A | 2-heptanol acetate | 5921-82-4 | ester | green | fatty, green, fruity (Banana) | x | **x** | x | x | x | x | **x** | |
| A | trans-ocimene | 3779-61-1 | acyclic monoterpene | herbal | sweet-herbal | x | x | x | **x** | **x** | **x** | x | all significantly higher in rainy season |
| B | alpha-ocimene | 502-99-8 | acyclic monoterpene | fruity | fruity-floral | x | x | x | **x** | **x** | **x** | **x** | all significantly higher in rainy season |
| B | linalool | 78-70-6 | acyclic terpene alcohol | floral | floral (lavender, lilly of the valley), Orange, waxy, rose | x | x | x | **x** | **x** | **x** | x | all significantly higher in rainy season |
| B | myrcene | 123-35-3 | acyclic monoterpene | spicy | herbaecous-woody, balsam, hemp | x | x | x | **x** | **x** | **x** | x | all significantly higher in rainy season |
| C | 2-methyl-3-buten-2-ol | 115-18-4 | unsaturaded alcohol | herbal | herbal, earthy, oily | x | **x** | x | **x** | **x** | x | x | all significantly higher in rainy season |
| C | 2-nonanone | 821-55-6 | ketone | fruity | fruity, sweet, waxy, soapy, coconut, green herbaceous | x | **x** | x | x | x | x | **x** | |
| C | 2-pentanone | 107-87-9 | ketone | fruity | banana-like with fermented woody nuance | x | x | x | x | **x** | x | x | |
| C | 2-heptanol | 543-49-7 | secondary alcohol | citrus | fresh, lemon grass, herbal, sweet, floral-fruity, green | x | **x** | x | x | x | x | **x** | |
| C | 1-phenylethyl acetate (gardenol) | 93-92-5 | ester | floral | Gardenia, dry, intensive, green, fresh, berry-fruity nuance | x | x | x | x | x | x | x | |
| C | 2-heptanone | 110-43-0 | ketone | cheesy | Cheese, fruity, green banana, cinnamon | x | **x** | x | x | x | x | **x** | |
| C | 2-pentanol | 6032-29-7 | secondary alcohol | fermented | alcoholic, fusel, fermented, musty with sweet white wine top notes | x | x | x | x | x | x | x | |

**x:** the volatile component was detected in relatively high amounts in this genotype (exact data available in Hegmann, 2015).

x (without [1] or [2]): the aroma compound was detected in both seasons

**Tab.4: Minor volatile aroma components detected in fruit pulps of different cocoa varieties**

D: Minor compounds with peak areas < 1,0E+08; E: Minor compounds with peak areas < 5,0E+07; F: Minor compounds with peak areas < 1,0E+07; G: Minor compounds with peak areas < 5,0E+06
Odour descriptions mentioned in Fig.1 and Tab.3 to Tab.6 derive from 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*) as well as Surburg and Panten (2006), Nozaki et al. (1997), Hui (2010), Lan-Phi et al. (2009). ANOVA and *post-hoc* Tukey HSD test for unequal N, with $\alpha = 0.05$ and $p < 0.01$.

| Category | Aroma compound | CAS-No. | Group | Odor | Odor description | Identified in | | | | | | | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | PMCT 58 | ICS-95 (TI) | CATIE-R1 | CATIE-R4 | CATIE-R6 | SCA-6 | EET 62 | |
| D | 2-octanol acetate | 2051-50-5 | ester | fruity | citrus (Orange) | | x | | | | | x | |
| D | 2-nonanol | 628-99-9 | secondary alcohol | fruity | waxy, green, creamy, citrus (Orange), cheesy | | **x** | | | | x | x | |
| E | 5-methyl-3-heptanone | 541-85-5 | ketone | herbal | herbal, sweet, oily | x | x | x | x | x | x | | detected in dry season only |
| E | linalyl acetate | 115-95-7 | ester | herbal | sweet, green, bergamot, spicy, woody, terpy and citrus nuance | x[1] | | x | **x** | **x** | **x** | x[2] | [1] rainy season<br>[2] dry season |
| E | 2-undecanone | 112-12-9 | ketone | fruity | waxy, fruity, pineapple nuances | | x | | | x[2] | **x** | | [2] dry season |
| E | trans-linalooloxide | 34995-77-2 | heterocyclic monoterpene derivative | floral | floral, earthy, slight bergamot | x | x[1] | x | **x** | x | x | | [1] rainy season |
| E | acetophenone | 98-86-2 | ketone | floral | orange blossom, sweet, cherry pit, marzipan and coumarinic, slight almond nutty and vanilla nuance | x | x | x | x | x | x | x | |
| E | 1-phenylethanol | 98-85-1 | secondary alcohol | floral | dry, roselike, slight hawthorn | x | x | x | x | x | x | | |
| E | alpha-bergamotene | 17699-05-7 | sesquiterpene | woody | warm, tealeaf, woody | | | x[2] | x | **x** | **x** | x[2] | [2] dry season |
| E | alpha-copaene | 3856-25-5 | tricyclic sesquiterpene | woody | woody, spicy, honey | | | x | x | x | x[2] | | [2] dry season |
| F | trans-beta-farnesene | 502-60-3 | sesquiterpene | fruity | green, fruity, citrus | | | x[2] | x | x | x | | [2] dry season |
| F | 2,3-butanediol diacetate | 1114-92-7 | diester | floral | honey | **x** | | x | x[2] | x | | | [2] dry season |
| G | epoxylinalool (pyranoid) | 14049-11-7 | cyclic terpene alcohol | floral | floral, honey | | x[2] | x[2] | **x** | x[2] | | x[2] | [2] dry season |
| G | cis-linalool oxide | 5989-33-3 | heterocyclic monoterpene derivative | earthy | earthy sweet woody | | | x | x | x | x | | detected in dry season only |

**x:** the volatile component was detected in relatively high amounts in this genotype (exact data available in Hegmann, 2015).
x (without [1] or [2]): the aroma compound was detected in both seasons

Differences between the aroma characters of the studied genotypes were primarily observed in aromas of lower concentrations. This allows the assumption that genotype-specific aroma bouquets derive from a mixture of various minor components. Some of them may be below the organoleptic threshold level, however, they could provide a new flavour quality in combination with other volatile aromas ("additive effects", Belitz, Grosch and Schieberle, 2009). Apparently, the identified aroma compounds are typical for cocoa pulps, however, they change during fruit ripening:

As shown in this study for the first time, aroma volatiles in fresh cocoa pulps vary strongly depending on the ripening stage of the fruits (Tab.5 and Tab.6). We observed that the complexity of low concentrated aroma components increases with increasing fruit ripeness.
In addition, minor components with odors described as green, herbal, vegetable, resp., decrease in favour of spicy, fruity, floral aroma components (Tab.5 and Tab.6). We assume that enzyme activities change throughout the fruit ripening process, however, so far neither cytological data nor information on the predominant key enzymes in *Theobroma cacao* L. are existent.

Volatile aroma compounds derive from different metabolic pathways. Aldehydes can originate from primary alcohols being formed during autoxidation[4] or enzymatic lipid peroxidation of saturated and unsaturated fatty acids (Belitz, Grosch and Schieberle, 2009) or, in the context of Strecker degradation, from amino acids (Afoakwa et al.2008). Terpenes and its derivatives, however, form either through the methyl D-erythritol 4-phosphate (MEP)-pathway or the mevalonate pathway. According to Rohmer (1999) mono- and diterpenes are synthesized via the MEP pathway occurring in plastids whereas sesquiterpenes derive from the mevalonate pathway in the cytosol. Sesquiterpenes were only detected in the genotypes CATIE-R1, CATIE-R4 and CATIE-6 as well as the control SCA-6. We conclude that terpenes play a decisive role in the aroma profiles of these genotypes whereas secondary alcohols, aldehydes, esters and ketones are the key components in the fruit pulp aroma of ICS-95 (T1) and PMCT-58, resp. (the latter primarily consisting of esters and ketones). Thus, our results support the hypothesis of Kadow et al. (2013) that differences between genotypes and their predominant aroma compounds originate from the different metabolic pathways of the substances.

A transfer of aroma volatiles from the fruit pulp into the cotyledons during fermentation and/or fruit ripening was already discussed by Eskes et al. (2009) and Kadow et al. (2013). Amores (2006, cited in Voigt and Lieberei, 2014) observed that the floral terpene alcohol linalool appeared in cotyledons on the third day of fermentation. In addition, Eskes et al. (2009) were able to show that pulp aromas of Cupuaçu (*Theobroma grandiflorum* Willd. Ex Spreng) and *Annona muricata*, previously added to the fermentation can be detected organoleptically in the final chocolate product.
The results of the present study combined with the previous findings allow the assumption of an interdependence between genotype, pulp aroma and flavour potential of the final chocolate product. We conclude that pulp properties as selection criteria for the development of new fine or flavour cocoa genotypes are useful once the transfer mechanisms of key components involved in flavour formation are clearly understood and identified (Voigt and Lieberei, 2014). For this, we suggest further research on genotype-specific characteristics such as testa-properties and pulp-seed-ratio.

[4] According to Belitz, Grosch and Schieberle (2009) the fatty acid pattern of *Theobroma cacao* L. with palmitic, stearic, oleic and linoleic acid in the ratio 25: 37: 34: 3, resp., largely excludes autoxidation.

**Tab.5: Minor volatile aroma components detected in fruit pulps of ripe and overripe fruits of different cocoa varieties**

Amounts: ≤ 0,3%; Description: 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)

| Aroma compound | CAS-No. | Group | Odor | Odor description | Identified in | | | | | | | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PMCT 58 | ICS-95 (TI) | CATIE-R1 | CATIE-R4 | CATIE-R6 | SCA-6 | EET 62 | |
| benzacetaldehyde | 122-78-1 | aromatic aldehyde | floral | honey, floral rose, powdery, fermented, chocolate with a slight earthy | | x | | | | | | |
| 2-octanol | 123-96-6 | secondary alcohol | spicy | fresh, spicy, green, woody, herbal, earthy | | x | | | | | | |
| beta-cubebene | 13744-15-5 | sesquiterpene | fruity | citrus, fruity, raddish | | | x | | | | | detected in dry season only |
| delta-cadinene | 483-76-1 | sesquiterpene | herbal | thyme, herbal, woody, dry | | | x | | | | | |
| alpha-gurjunene | 489-40-7 | sesquiterpene | woody | wood, balsam | x | | x | | | | | detected in dry season only |
| nonanal | 124-19-6 | aldehyde | floral | waxy, aldehydic, citrus, with slightly green lemon peel and cucumber | x | x | | x | | | | |
| benzyl acetate | 140-11-4 | ester | floral | sweet, fruity and floral | x | | x | x | x | | | |
| 2-octanone | 111-13-7 | ketone | earthy | ketonic, blue and parmesan cheese-like, earthy, dairy nuances | | x | | | x | | | |

**Tab.6: Minor volatile aroma components detected in fruit pulps of unripe fruits of different cocoa varieties**

Amounts: ≤ 0,3%; Description: 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)

| Aroma compound | CAS-No. | Group | Odor | Odor description | Identified in | | | | | | | Comment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | PMCT 58 | ICS-95 (TI) | CATIE-R1 | CATIE-R4 | CATIE-R6 | SCA-6 | EET 62 | |
| heptanal | 111-71-7 | aldehyde | green | green, herbal, fruity, fatty | | x | | x | | | | |
| hexanal | 66-25-1 | aldehyde | green | green, fatty, leafy, vegetative, fruity, woody nuance | | x | | x | | | | |
| trans-2-cis-6-nonadienal | 557-48-2 | aldehyde | green | green, cucumber, melon, fatty, vegetative | | | | x | | | | |
| trans-2-nonenal | 18829-56-6 | aldehyde | green | green, cucumber, aldehydic, fatty with citrus nuance | | | | x | | | | |
| 3-methyl-1-butanol | 123-51-3 | alcohol | fermented | alcoholic, pungent, etherial, cognac, fruity, banana, molasses | | | | x | | | | |
| 3-octanol | 589-98-0 | alcohol | earthy | earthy, mushroom, dairy, creamy, waxy, slight fermented, green | | | | x | | | | |
| 1-hexanol | 111-27-3 | primary alcohol | herbal | pungent, etherial,fruity, alcoholic, sweet with green top note | x | | | | | | | |
| trans-2-octenal | 2548-87-0 | aldehyde | green | citrus | | x | | x | | | | |
| trans-2-hexenal | 6728-26-3 | aldehyde | green | sharp, penetrating fresh leafy, green, fruity, herbal and spicy nuances | x | | | x | | | | |
| cis-3-hexenyl acetate | 3681-71-8 | ester | green | fresh, green, sweet, fruity with apple, pear, melon nuances | | | x | | x | | | |
| 2-pentyl-furan | 3777-69-3 | furan derivative | green | fruity, green, earthy, beany, vegetable nuances | x | **x** | x | x | x | x | x | |
| cis-3-hexenol | 928-96-1 | unsaturaded alcohol | green | green, grassy, melon rind, pungent freshness | **x** | x | x | x | x | x | x | |

**x:** the volatile component was detected in relatively high amounts in this genotype; x (without [1] or [2]): the aroma compound was detected in both seasons (exact data available in Hegmann, 2015).

## 5. Conclusion

Various volatile aroma compounds identified in commonly known fine flavor cocoas (EET 62, SCA 6) were detected in fresh fruit pulps of the new cocoa clones CATIE-R1, CATIE-R4, CATIE-R6, PMCT-58 and ICS-95 (T1). This allows their classification as Fine or Flavour cocoas. The pulp of each clone has a genotype-specific aroma character, due to differences in the predominant chemical compounds.

In addition, this study shows that both,

a) the prevailing climate in the phase of fruit ripening and
b) the ripening status of the fruit

play a decisive role in the change of the fruit pulp's aroma composition and aroma intensity. The interdependence of pulp quality and the organoleptic character of the end product allows to postulate that both, aroma intensity and aroma composition of fine chocolates may vary depending on the raw cocoa being used from main crop and mid-crop, resp.. A certain but small amount of cocoa from overripe fruits in the fermentation mass may enhance the fine aroma character of the chocolate. With correct post-harvest management (Hegmann, 2015) the studied CATIE-selections can result high quality fine flavor-raw cocoa. However, inadequate fermentation and/or drying can easily destroy the fine aroma.

To date there has been no economically significant production of chocolates made out of one single genotype. However, as the present study shows, fine flavour potential and aroma profiles vary greatly depending on the given genotype. "True-to-type"-products or blends made out of specific cocoa varieties could become an interesting component for chocolate manufacturers focused on exclusivity and niche markets.

Further investigations of the biochemical background are needed in order to understand the detailed mechanisms and transfer processes involved in the fine aroma formation of *Theobroma cacao* L.

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