



Sugar profile and volatile aroma composition in fermented dried beans and roasted nibs from six controlled pollinated Cameroonian fine-flavor cocoa (*Theobroma cacao* L.) hybrids

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ABSTRACT

The quality of chocolate products proven by consumer acceptability mostly depends on cocoa volatile compounds. In fact, aroma profile of cocoa or chocolate is correlated to cocoa genotype, culture conditions, post-harvest practices, and chocolate manufacturing. To the best of our knowledge, no study has been published on both sugars and aromas of controlled cocoa hybrids from Cameroon. The present study aimed at analyzing biochemical and volatile composition of six hand-pollinated cocoa hybrids belonging to different traditional groups. Specifically, the sugar profile was obtained by high performance ion chromatography-electrochemical detector (HPIC-ED) and aroma composition was analyzed using headspace solid phase micro-extraction coupled with gas chromatography-mass spectrometry (HS-SPME/GC-MS) on fermented dried beans and roasted nibs. The content of sugars was variable among hybrids and fructose showed the highest concentration in all hybrids in the two matrices. Roasting decreased sugar content. However, the decrease in sugar content seemed genotype dependent. A total of 48 volatile aromas were classified in six groups according to their odor description (fruity, floral, chocolate/nutty, buttery/creamy, undesirable, and unknown). Fruity flavors were not specific to a given chemical group. However, floral volatiles were specific to terpenes, chocolate/nutty to pyrazines, buttery/creamy to lactones and undesirable to phenols and acids. Principal component analysis (PCA) showed specificity of each hybrid and the hierarchical cluster analysis (HCA) Heatmap presented four distinct groups. SCA12 × ICS40 with the highest pyrazine concentration was associated with important content of sugars. Roasting of nibs reduced total volatiles and particularly fruity volatiles whereas other flavor groups moderately increased. In fact, some volatiles such as 2,3-butanediol, 2-nonanol, linalool, β-myrcene, cis/trans-β-ocimene, 2-nonanone, 2-heptanol, methyl acetate, acetophenone could be used as markers in separating traditional cocoa groups in Cameroon.

1. Introduction

Cocoa (*Theobroma cacao* L.) is a perennial crop which grows in tropical and subtropical zones of the globe. Over 5.4 million tons of cocoa were produced during the 2021/2022 crop year worldwide. Ivory Coast, Ghana, Ecuador, Cameroon, Nigeria, Brazil, and Indonesia alongside other 58 countries around the world (ICCO, 2022). Cocoa is

the main ingredient of chocolate and confectionery industries. Many genotypes are encountered and hand-pollination between cocoa clones leads to controlled hybrid populations with either disease tolerant (Djoko Kouam et al., 2022) or agronomic and some physicochemical parameters performance or both (Effa, Akoa, Ondobo, & Djocgoue, 2016; Akoa, 2021b). According to the origin and the morphological criteria, three major groups of cocoa are known: Criollo (fine cocoa,

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5%), Forastero (bulk cocoa >80%) and Trinitario (hybrids of the two previous groups with some interesting aromas) (Aprotosoiaie, Luca, & Miron, 2016). Specifically, pure/perfect Trinitario hybrid production is limited by many incompatibility cases reported between clones (Akoa, Effa Onomo, Manga Ndjaga, Ondobo, & Djocgoue, 2021; Looor Sol-órzano, 2007). However, breeding between Trinitario and Forastero shown promising results (Akoa, Effa Onomo, et al., 2021).

Cameroon is the largest African producer (~20%) of Trinitario cocoa (Aprotosoiaie et al., 2016) with SNK (Selection of Nkoemvone) and ICS (Imperial College Selection) the most important subgroups. The SNK clones known as local Trinitario (LT) are the most representative in Cameroon. ICS clones were selected in Trinidad. Akoa, Djoko Kouam, et al. (2021) highlighted the important content of procyanidin C and caffeine in SNK and ICS40 cocoa hybrids respectively. Another cocoa found in Cameroon is Scavina (Forastero). It is known for its floral aroma in fruit pulp and fresh cotyledons (Kadow, Bohlmann, Philips, & Lieberei, 2013). Moreover, Scavina 12 cocoa pods with an important bean number proved to be richer in (–)-epicatechin and more precocious (Akoa, Effa Onomo, et al., 2021; Ondobo, 2014, p. 148).

The importance of aroma compounds in foods has been revealed by many authors. More than 600 volatile compounds belonging to many chemical groups were identified in cocoa and cocoa by-products (Aprotosoiaie et al., 2016). According to the aromatic potential, cocoa is classified in two categories namely fine/flavor cocoa and bulk/ordinary cocoa. Fruity/floral volatiles have been associated to fine/flavor cocoa. According to several findings, floral or fruity volatiles were associated to important amounts of secondary alcohol or their esters, terpenoids, and ketones. In cocoa or cocoa products, some aromas can describe the state of cocoa beans. Then, varietal/environmental aroma present in mature fruits and describe the genotype and the conditions of growing. Fermenters are synthesized during fermentation by microorganisms. Finally, thermal volatiles found in roasted cocoa describe thermal treatment affecting cocoa process. Rottiers et al. (2019) noted an increase in concentration of some varietal volatiles during fermentation. Moreover, Kongor et al. (2016) mentioned that cocoa genotypes are significantly related to their chemical composition. However, post-harvest practices (fermentation and drying) can change the chemical composition of cocoa beans. Considered as a key step, it is during fermentation that aroma precursors mainly sugars and amino acids are generated (Aprotosoiaie et al., 2016; Jinap, Siti, & Norsiat, 1994). Practically, glucose and fructose derived from sucrose hydrolysis were found to be the two major reducing sugars in fermented cocoa beans (Rottiers et al., 2019; Sande Santos et al., 2020). Roasting induces biochemical reactions in cocoa beans producing the characteristic of chocolate flavor. Presently, there is a lack of data on biochemical and volatile aroma composition of controlled Trinitario and Scavina cocoa hybrids from Cameroon. This work aimed at characterizing sugars and

volatile compounds in fermented dried beans and roasted nibs from six controlled cocoa hybrids using HPIC-ED and HS-SPME-GC-MS respectively.

2. Materials and methods

2.1. Raw materials

The technique of hand-pollination, as described by Akoa, Effa Onomo, et al. (2021), was used to produce six hybrids of cocoa from 9 clones: (♀)SNK16 × (♂)T60/887, (♀)ICS40 × (♂)UPA134, (♀)SNK10 × (♂)IMC67, (♀)SNK109 × (♂)T79/467, (♀)ICS40 × (♂)SCA12, and (♀)SCA12 × (♂)ICS40 during two consecutive crop years (2019 and 2020). Postharvest practices have been carried out according to the previous authors. The characteristics of the different hybrids were summarized in Table 1.

2.2. Sample preparation

Fermented dried beans were broken using a specific cocoa breaker (CAPCO, United Kingdom) and deshelled using an electromechanical apparatus (winover, CAPCO, United Kingdom) to obtain nibs. These nibs were divided in two equal parts. One part was roasted at 120 °C for 20 min 500 g of dried cocoa nibs (or roasted nibs) were put in liquid nitrogen and ground using a waring blender (Moulinex, the original grinder, England), to obtain cocoa flour which was stored at –20 °C until analysis. Approximately 2 g of cocoa powder were introduced into vial (10 mL), 100 µL of internal standard of 1-butanol (0,623 g/L, Sigma) were added and the vial was sealed with a septum cap.

2.3. HS-SPME protocol







The extraction of three technical repetitions was performed using a 50/30 µm divinylbenzene/carboxene/polydimethylsiloxane fiber (DVB/CAR/PDMS, Supelco), and using the headspace solid phase micro-extraction (HS-SPME) technique as previously described by Hamdouche et al. (2019). Additionally, the fiber was conditioned at 250 °C for 3 min and was exposed to the sample headspace at 50 °C for 45 min.

2.4. Analytic conditions of the gas chromatography process

The chromatographical conditions applied were adopted from Hamdouche et al. (2019) with slight modifications. The volatile compounds extracted from cocoa powder were analyzed using an Agilent 6890N gas chromatography-mass spectrometer (GC-MS) in automatic injection mode on a polar capillary column DB-WAX, 60 m length × 0.25 mm internal diameter × 0.25 µm film thickness (Agilent, CA, USA). The

Table 1

General characteristics of the different cacao hybrids (Akoa, Effa Onomo, et al., 2021, Niemanak, Rohsius, Elwers, Ndoumou, & Lieberei, 2006).

Hybrids	Group	Pod length (cm)	Pod weight (g)	Bean number per pod	Weigh of fresh bean (g)	Susceptibility to black pod disease	Photo
SCA12xICS40 (F12)	LAFxT	19.66 ± 0.64	559.74 ± 53.73	47.60 ± 1.43	2.65 ± 0.39	Early tolerant	
ICS40xSCA12 (F40)	TxLAF	27.07 ± 1.37	727.65 ± 60.01	38.90 ± 1.20	4.88 ± 1.20	Fairly susceptible	
ICS40 × UPA134 (F13)	TxUAF	25.33 ± 0.42	781.84 ± 58.20	38.60 ± 3.56	4.28 ± 0.36	Fairly susceptible	
SNK10 × IMC67 (F17)	LTxUAF	23.34 ± 1.42	379.56 ± 12.07	42.32 ± 1.15	3.87 ± 0.49	Early tolerant	
SNK16 × T60/877 (F16)	LTxUAF	22.87 ± 0.71	633.53 ± 78.67	45.42 ± 2.32	3.72 ± 0.78	Tolerant	
SNK109 × T79/467 (F14)	LTxUAF	24.96 ± 0.73	574.43 ± 51.43	47.02 ± 4.45	3.98 ± 1.01	Early tolerant	

SCA: Scavina, ICS : Imperial College Selection, UPA : Upper Amazone, SNK : Selection of Nkoemvone, IMC : Iquitos Mixed Collection, T: Tafo, LAF: Lower Amazonian Forastero, T: Trinitario, UAF: Upper Amazonian Forastero, LT: Local Trinitario.

GC-MS conditions were established in CIRAD laboratory. The injection temperature was 250 °C. The GC oven temperature was initially set at 40 °C for 5 min, increased to 140 °C at a rate of 2 °C/min and then increased at a rate of 10 °C/min to 250 °C. The carrier gas was hydrogen at 1 mL/min. Splitless injection mode was used at 250 °C for 2 min. The selective mass detector was a quadrupole (Hewlett Packard, Model 5973N) with an electronic impact ionization system at 70 eV with the temperature source at 230 °C.

2.5. Volatile compounds identification and quantification

Volatile compounds were identified as described by Assi-Clair et al. (2019) with slight modifications, using three criteria: (i) by comparison of the retention index with CIRAD aromatic database, (ii) by comparison of Kovats indices determined after injection of a series of n-alkane homologues (C8–C20, KI calculated), with the Kovats indices from literature (KI literature) with NIST17 library and, (iii) whenever possible, the identification was confirmed using pure standards of major volatiles in each flavor note (2,3,5-trimethyl pyrazine, acetoin and linalool, Sigma-Aldrich, Darmstadt, Germany; acetic acid, AP Chemicals NV, Belgium; ethyl acetate, Fisher Scientific, Strasbourg, France; benzaldehyde, labbox, Paris, France). Peak areas were used for quantification of compounds using the MSD Chemstation software (version E.02.02.1431, Agilent Technologies) with 1-butanol (0,623 g/L, Sigma) as internal standard.

2.6. Sugars analysis by HPIC-ED

The method adopted for sugar extraction was proposed by Bianco-lillo et al. (2021) with slight modifications. An average of 100 mg of cocoa powder were mixed with 1.5 mL of acidified pure water (0.002 M H₂SO₄). The solution was put into the Ultrason (Fisher Scientific, USA) for 15 min at 70 °C. The mixture was homogenized on a laboratory vortex (Legallais, France) for 2 h at 60 rpm, 22 °C and followed by centrifugation for 10 minutes at 15000 g (Sigma, France). The supernatant solution was filtered through a 0.45 µm syringe membrane filter (Sortorius RC Minisart™, Thermo Fisher Scientific, England) and subjected to high performance ion chromatography (HPIC, ICS-5000 + DC, Thermo Fisher Scientific, USA) analysis.

Sugars were separated by anion exchange chromatography in an isocratic mode (Dionex SP) as described by Shi et al. (2019) with slight modifications. 10 µL of each sample were automatically injected onto the column CarboPack PA210 Fast (4 × 150 mm, 4 µm) supported by a pre-column CarboPack PA210 Fast (4 µm) (Thermo Fisher Scientific, USA) established at 30 °C using a flow rate of 0.8 mL/min. The chromatography system consisted of an automatic injector (Dionex AS-AP), an eluent generator (Dionex ICS-5000 + EG) and an isocratic pump (Dionex SP). The eluent used in the mobile phase was KOH aqueous solution in three different concentrations. The gradient of analysis was established as followed: KOH 12 mM (eluent A) for 20 min, KOH 40 mM (eluent B) for 10 min and KOH 100 mM (eluent C) for 10 min. Entire system is controlled by a PC, configured with Thermo Dionex Chromeleon 7.1.2 Chromatography Data System which provides complete instrument control, data acquisition, and data management. The identification of each sugar was based on retention times of commercial standards used. The retention times of the various sugars were determined by recording the electrochemical detector (ICS-5000 + ED). Sugars were quantified with the peak area of each sugar in samples and calibration curve of standard solutions. Extract injection was repeated twice.

2.7. Statistical analysis

Both Significance differences in the volatile component profiles of the samples, tested by one-way analysis of variance (ANOVA) and principal component analysis (PCA, used to study the relationships

between the samples in terms of their flavor profiles) were performed using XLSTAT 2022 (Addinsoft, Paris, France) for Windows. The Venn diagram (complete clustering method, ward distance and Euclidian metric) was obtained using R Studio version 1.4.1103 for macOSX10_14_6. The HCA-Heatmap of volatile compounds in fermented dried beans from different hybrids was generated using Python (Jupyterlab) version 3.0.14 for macOSX10_14_6.

3. Results and discussion

3.1. Reducing and non-reducing sugars

The sum of individual sugars showed in Fig. 1 (A & B) varied considering the matrix and the genotype of cocoa. Among the three reducing sugars identified (glucose, fructose, and galactose), the content of fructose was more important regardless of genotype and matrix (Fig. 1A). It is well known that after fermentation and/or drying, the content of fructose is far higher than that of glucose. In fact, during fermentation, as sucrose is hydrolyzed to glucose and fructose, the microorganisms preferably metabolize glucose instead of fructose (Reineccius, Andersen, Kavanagh, & Keeney, 1972). The highest content of fructose was recorded by the hybrid SCA12 × ICS40 (3.96 ± 0.21 g/100 g) whereas the lowest content was obtained with SNK10 × IMC67 (3.01 ± 0.12 g/100 g) in fermented dried beans (Fig. 1A). The content of fructose was nine to twelve times more important than that of glucose and five to seven times more than that of sucrose in all fermented dried beans. The ratio of fructose-glucose is usually considered as an indicator of fermentation. Some findings highlighted that fructose-glucose ratio could play a key role in channeling the outcome of the Maillard reaction after subsequent roasting of fermented dried beans (Beckett, 2009). In fact, Authors found that a 7 day-preconditioning period increased the content of fructose in fermented dried cocoa beans (Hinneht et al., 2018). Like for fructose, the contents of glucose and sucrose were higher in SCA12 × ICS40 hybrid. The lowest content for glucose (0.26 ± 0.04 g/100 g) was found in ICS40 × SCA12 fermented dried beans. Pre-conditioned (7 days) cocoa pods can increase the content of sucrose in different genotypes. But this increase seemed to be in the dependency of the pulp physical property thus genetic identity. Furthermore, the content of sucrose was positively correlated to the organic acid content and the fermentation index (FI) of cocoa beans (Biehl et al., 1989). The fermented dried beans of SCA12 × ICS40 showed the lowest FI among samples (Akoa, Effa Onomo, et al., 2021). Moreover, some cocoa varieties required proper fermentation practice to release more reducing sugars from sucrose (Niemenak et al., 2021). The content of glucose was used as an indicator in separating the three groups of cocoa hybrids. Results showed that the content of glucose was lower in ICS40 fermented dried beans. The three hybrids of SNK (SNK16 × T60/877, SNK109 × T79/467 and SNK10 × IMC67) showed practically the same amount of glucose and slightly below the top content which was obtained with fermented dried beans from SCA12 × ICS40 hybrid. In contrast with fructose, the content of galactose was marginal in dried fermented beans from six different genotypes considered in this study. Other findings reported the presence of supplementary sugars in cocoa (Niemenak et al., 2021; Reineccius et al., 1972). In this study, many reducing or non-reducing sugars were identified namely verbascose, stachyose, maltose, and raffinose. The content of raffinose (0.61 ± 0.01 g/100 g) and maltose (0.16 ± 0.03 g/100 g) were also higher in SCA12 × ICS40 hybrid fermented dried beans (Fig. 1B) than in other hybrids.

A remarkable reduction in sugar content was observed during roasting. This decrease in fructose content was the most important. The reduction in sugar content mainly fructose and glucose during roasting is well known. In fact, it is during this step that Maillard reactions and Strecker degradations take place (Afoakwa, Paterson, Fowler, & Ryan, 2008; Ge et al., 2021). These reactions utilize reducing sugars (fructose and glucose) with amino acids/short peptides to produce volatile aromas in roasted foods. The decrease in reducing sugars was significant

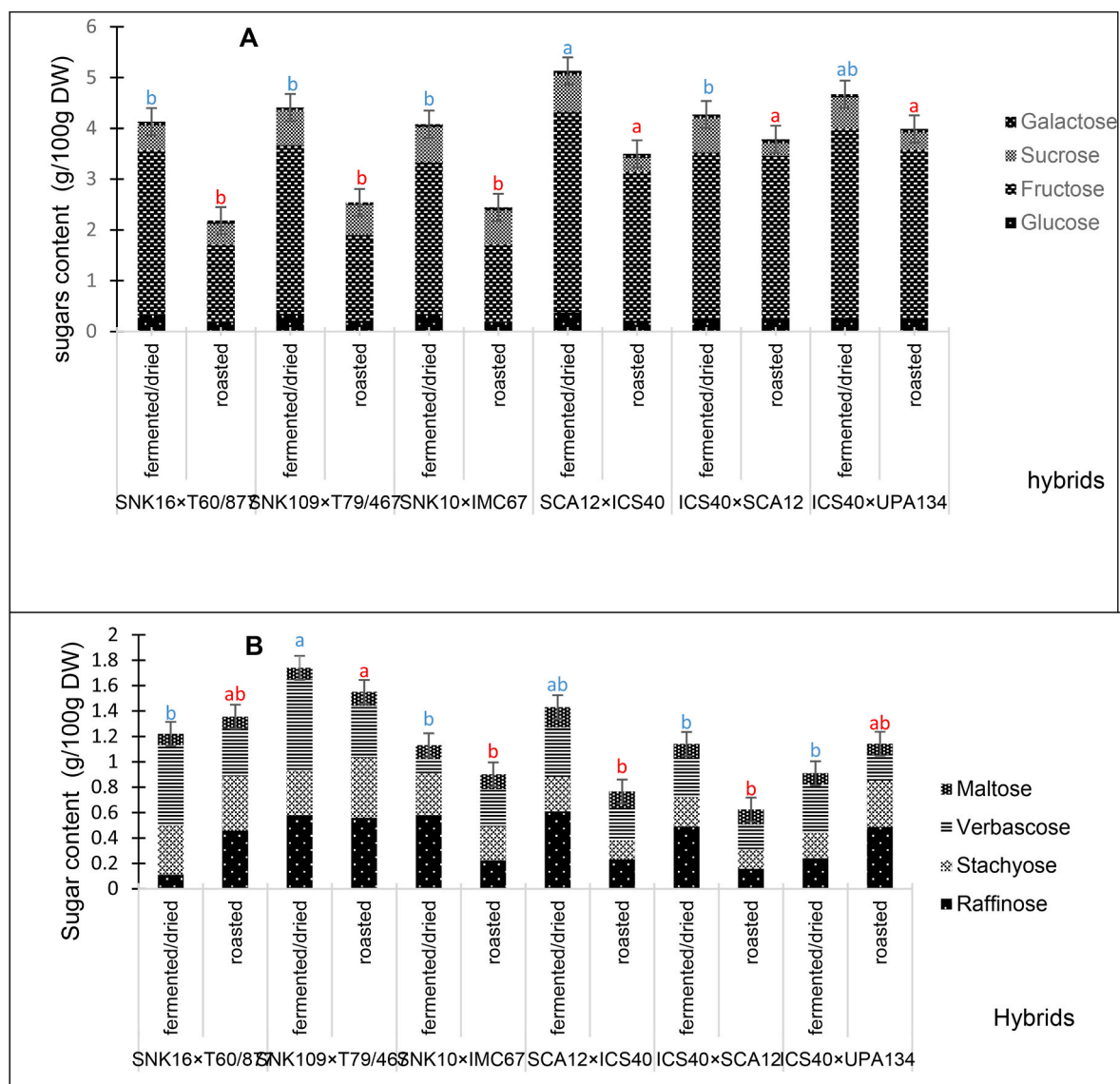


Fig. 1. Principal sugar compounds identified by HPIC-ED fermented dried and roasted beans of six hybrids of cacao. (A) Sucrose and 3 reducing sugars. (B) Maltose and 3 non-reducing sugars. Fermented dried beans Roasted nibs.

in SNK hybrids: 37.38, 44.82, and 48.81% for SNK109 × T78/467, SNK16 × T60/887 and SNK10 × IMC67 respectively. Indeed, a reduction of 37.36% was observed for SCA12 × ICS40. Inversely, the ICS40 hybrids showed the lowest content in sugars from roasted nibs. Approximately 9.69% (ICS40 × UPA134) and 18.67% (ICS40 × SCA12). The reduction in sugar content in some cocoa hybrids during roasting may depend on the sugar type and the genotype of cocoa. De Brito et al. (2000) roasted three days fermented dried Forastero beans from Brazil and obtained a reduction from 1.27 mg/g to 0.50 mg/g and from 0.28 mg/g to 0.27 mg/g for fructose and glucose respectively. Concerning the four other sugars in Fig. 1B, roasting was associated with a decrease in sugar content in four hybrids. In SNK16 × T60/887 and ICS40 × UPA134 hybrids, we noted an increase of raffinose content. Indeed, we observed a moderate increase in verbascose content in SNK16 × T60/887 roasted nibs and a stagnation of raffinose content. Therefore, the decrease of these sugars was not statistically significant in SNK16 × T60/887 during roasting (Fig. 1B).

3.2. Volatile compounds in fermented dried and roasted nibs

The GC-MS analysis showed volatile composition of the fermented dried beans and roasted nibs. A total of 48 volatile components was retained for this study including acids, esters, alcohols, ketones, aldehydes, pyrazines, terpenes, lactones, pyrroles, and furans. Volatile compounds were classified into fruity (15), floral (11), chocolate/nutty (10), buttery/creamy (3), undesirable (4) and others (5) volatiles according to their general odor characteristic (Table 2). Practically, all these chemical groups are common in cocoa.

3.2.1. Fruity volatiles

Fifteen compounds were identified as fruity volatiles according to their odor description. Results showed up to ten compounds were predominant according to their concentration. These volatile compounds differed from one hybrid to another although some hybrids showed similar values. Fermented dried beans recorded the highest content of methyl acetate in fruity volatiles in all hybrids. Its concentration was most important in ICS and SCA12 hybrids than in SNK ones. Besides this compound, the hybrid SCA12 × ICS40 was dominated by ethyl acetate

Table 2

Average concentrations (mean \pm SD) of volatile compounds ($\mu\text{g/g}$ nibs 1-butanol equivalents) identified in fermented dried cocoa beans by HS-SPME-GC-MS ($n = 3$) and odor description.

N°	Volatile compounds	SCA12 \times ICS40	SNK109 \times T79/467	ICS40 \times UPA134	ICS40 \times SCA12	SNK16 \times T60/887	SNK10 \times IMC67	Chemical group	Odor description	KI lit.	KI cal.
1	2-nonanol	0.33 \pm 0.10 ^b	1.09 \pm 0.25 ^a	0.36 \pm 0.12 ^b	0.35 \pm 0.04 ^b	1.07 \pm 0.13 ^a	0.93 \pm 0.11 ^a	Alcohol	Fruity citrus, Waxy, fatty, spicy (π)	–	1521 \pm 11
2	2,3-butanediol	0.20 \pm 0.01 ^a	0.01 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	0.00 \pm 0.00 ^b	Alcohol	Fruity, creamy, buttery (θ)	1492–1582	1533 \pm 14
3	2-heptanol	24.08 \pm 1.56 ^c	34.11 \pm 4.38 ^b	6.40 \pm 0.39 ^d	7.13 \pm 0.51 ^d	51.54 \pm 4.61 ^a	48.79 \pm 7.46 ^a	Alcohol	Fruity, lemon grass, floral, fresh, sweet, herbal, green (β)	1326	1320 \pm 9
4	2-heptanol acetate	18.02 \pm 0.95 ^a	5.95 \pm 1.59 ^b	2.74 \pm 0.29 ^b	3.72 \pm 0.31 ^b	18.42 \pm 0.21 ^a	17.13 \pm 1.36 ^a	Ester	Fruity (α)	1250	1255 \pm 3
5	2-heptanone	0.31 \pm 0.04 ^{bc}	0.19 \pm 0.04 ^c	0.59 \pm 0.06 ^{ab}	0.70 \pm 0.04 ^a	0.41 \pm 0.01 ^{bc}	0.50 \pm 0.13 ^{ab}	Ketone	Fruity, sweet, coconut, spicy, herbal, woody (β)	1181	1182 \pm 8
6	2-nonanone	4.98 \pm 0.4 ^{bc}	9.72 \pm 2.31 ^a	3.06 \pm 0.27 ^c	2.12 \pm 0.18 ^c	8.79 \pm 1.60 ^{ab}	9.97 \pm 2.72 ^a	Ketone	Fruity, sweet, waxy, green herbaceous, coconut like (θ)	1389	1390 \pm 7
7	2-pentanol	9.78 \pm 0.35 ^d	26.09 \pm 1.15 ^a	20.01 \pm 2.58 ^b	15.78 \pm 1.37 ^c	9.70 \pm 0.15 ^d	8.34 \pm 0.74 ^d	Alcohol	Green, fruity, sweet, pungent, plastic (θ)	1122	1119 \pm 10
8	2-pentanol acetate	6.22 \pm 0.37 ^{bc}	7.13 \pm 0.56 ^b	10.26 \pm 1.08 ^a	9.67 \pm 0.24 ^a	5.07 \pm 0.22 ^{cd}	3.93 \pm 0.30 ^d	Ester	Fruity, orange, tropical (α)	1050	1069 \pm 5
9	butyl acetate	20.32 \pm 2.64 ^{abc}	27.68 \pm 4.70 ^a	19.48 \pm 2.08 ^{bc}	23.95 \pm 2.98 ^{ab}	9.39 \pm 0.45 ^d	14.53 \pm 2.07 ^{cd}	Ester	Sharp, etherial, diffusive, fruity banana (δ)	1075	1074 \pm 8
10	ethyl acetate	48.70 \pm 2.24 ^a	49.48 \pm 11.68 ^a	24.74 \pm 6.67 ^b	15.06 \pm 1.06 ^b	17.81 \pm 0.94 ^b	9.56 \pm 2.24 ^b	Ester	Fruity, aromatic (ϕ)	872	888 \pm 8
11	ethyl isopentanoate	0.23 \pm 0.01 ^{ab}	0.16 \pm 0.03 ^b	0.26 \pm 0.02 ^a	0.15 \pm 0.01 ^b	0.17 \pm 0.04 ^b	0.21 \pm 0.01 ^{ab}	Ester	Sweet, diffusable, fruity, sharp, apple, green and orange (γ)	1072	1068 \pm 8
12	isoamyl acetate	9.23 \pm 0.43 ^c	50.54 \pm 4.52 ^a	19.45 \pm 0.33 ^b	24.58 \pm 1.59 ^b	5.42 \pm 0.58 ^c	5.30 \pm 0.23 ^c	Ester	Banana, fruity (α)	1118	1122 \pm 7
13	isoamyl alcohol	0.69 \pm 0.01 ^c	7.89 \pm 0.28 ^a	1.36 \pm 0.08 ^b	1.79 \pm 0.31 ^b	0.40 \pm 0.02 ^c	0.72 \pm 0.17 ^c	Alcohol	Banana, fruity, fermented, cognac (α)	1214	1209 \pm 9
14	isobutyl acetate	0.14 \pm 0.01 ^a	0.07 \pm 0.01 ^b	0.01 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.15 \pm 0.02 ^a	0.15 \pm 0.02 ^a	Ester	Fruity (α)	1008	1012 \pm 8
15	methyl acetate	541.66 \pm 98.33 ^a	312.54 \pm 143.99 ^b	566.15 \pm 47.90 ^a	623.77 \pm 53.63 ^a	207.51 \pm 29.90 ^c	185.50 \pm 24.49 ^c	Ester	Fruity (σ)	813	828 \pm 6
	Fruity	684.89 \pm 137.45^a	532.65 \pm 78.56^{ab}	674.87 \pm 144.44^a	728.77 \pm 159.35^a	335.85 \pm 52.89^b	305.56 \pm 47.34^b				
16	2-phenethyl acetate	3.24 \pm 0.35 ^{bc}	25.26 \pm 5.37 ^a	8.48 \pm 2.85 ^b	5.28 \pm 1.35 ^{bc}	1.21 \pm 0.14 ^c	1.02 \pm 0.01 ^c	Ester	Floral, honey (α)	1810	1813 \pm 15
17	2-phenyl ethanal	3.47 \pm 0.63 ^{ab}	5.86 \pm .96 ^a	3.04 \pm 0.72 ^b	2.56 \pm 0.54 ^b	1.77 \pm 0.26 ^b	1.65 \pm 0.31 ^b	Aldehyde	Honey, floral rose, sweet, powdery, chocolate (γ)	1634	1640 \pm 13
18	2-phenylethanol	7.06 \pm 1.03 ^c	46.88 \pm 4.98 ^a	25.33 \pm 5.75 ^{ab}	16.14 \pm 2.29 ^b	3.82 \pm 1.21 ^c	5.57 \pm 1.79 ^c	Alcohol	Floral, rose (λ)	1891	1906 \pm 15
19	acetophenone	6.94 \pm 0.44 ^b	12.74 \pm 3.58 ^a	5.97 \pm 1.93 ^b	4.95 \pm 0.48 ^b	8.55 \pm 0.96 ^{ab}	8.96 \pm 2.65 ^{ab}	Ketone	Floral, fruity (λ)	1642	1647 \pm 13
20	benzyl alcohol	0.61 \pm 0.04 ^b	1.10 \pm 0.30 ^a	0.43 \pm 0.06 ^{bc}	0.65 \pm 0.02 ^b	0.09 \pm 0.01 ^c	0.33 \pm 0.09 ^{bc}	Alcohol	Floral, rose, phenolic, balsamic (θ)	1872	1870 \pm 14
21	cis/trans- β -ocimene	15.16 \pm 0.57 ^a	0.00 \pm 0.00 ^b	0.13 \pm 0.01 ^b	0.26 \pm 0.02 ^b	0.24 \pm 0.04 ^b	0.52 \pm 0.06 ^b	Terpenes	Sweet, floral, earthy, woody (α)	1241	1250 \pm 6
22	cis-linalool oxide	1.53 \pm 0.08 ^a	0.87 \pm 0.20 ^b	1.25 \pm 0.08 ^a	1.32 \pm 0.18 ^a	0.54 \pm 0.01 ^{bc}	0.48 \pm 0.07 ^c	Terpenes	Sweet, woody, floral, creamy, slight earthy (α)	1423	1444 \pm 19
23	ethyl phenyl acetate	1.01 \pm 0.05 ^{bc}	2.80 \pm 0.67 ^a	1.35 \pm 0.44 ^b	0.62 \pm 0.12 ^{bc}	0.20 \pm 0.04 ^c	0.26 \pm 0.08 ^c	Ester	Floral honey, rosy with	–	1783 \pm 10

(continued on next page)

Table 2 (continued)

N°	Volatile compounds	SCA12 × ICS40	SNK109 × T79/467	ICS40 × UPA134	ICS40 × SCA12	SNK16 × T60/887	SNK10 × IMC67	Chemical group	Odor description	KI lit.	KI cal.
24	linalool	3.82 ± 0.10 ^a	0.11 ± 0.02 ^c	0.42 ± 0.02 ^b	0.37 ± 0.13 ^{bc}	0.35 ± 0.06 ^{bc}	0.61 ± 0.19 ^b	Terpenes	balsamic dark chocolate and cocoa notes (ε) Citrus, floral, woody, green blueberry (α)	1537	1547 ± 7
25	methyl phenyl acetate	11.03 ± 0.29 ^a	1.78 ± 0.48 ^d	4.72 ± 0.89 ^c	6.47 ± 0.95 ^b	1.79 ± 0.50 ^d	2.46 ± 0.24 ^d	Ester	sweet, floral, fruity, green (π)	1747	1750 ± 7
26	β-myrcene	17.02 ± 0.88 ^a	0.00 ± 0.00 ^c	0.49 ± 0.09 ^{bc}	0.65 ± 0.12 ^{bc}	0.40 ± 0.01 ^{bc}	1.22 ± 0.39 ^b	Terpenes	Balsamic, must, spicy, sweet (ω)	1159	1161 ± 7
	Floral	70.89 ± 5.68^b	97.4 ± 14.77^a	51.61 ± 7.36^{bc}	39.27 ± 4.73^c	18.96 ± 2.51^d	23.08 ± 2.74^d				
27	2,3,5,6-tetramethyl-pyrazine	207.69 ± 9.53 ^a	27.43 ± 8.87 ^c	116.92 ± 5.49 ^b	113.96 ± 4.33 ^b	43.87 ± 5.49 ^c	29.10 ± 9.00 ^c	Pyrazine	Chocolate, cocoa, coffee (μ)	1489	1469 ± 7
28	2,3,5-trimethyl-pyrazine	27.90 ± 1.73 ^a	3.19 ± 1.07 ^d	16.22 ± 0.82 ^b	6.94 ± 0.28 ^{cd}	14.37 ± 1.53 ^b	10.81 ± 3.10 ^{bc}	Pyrazine	Cocoa, rusted nuts, peanut (μ)	1408	1402 ± 8
29	2,3-dimethyl-pyrazine	5.15 ± 0.39 ^a	0.83 ± 0.21 ^c	2.63 ± 0.12 ^b	1.26 ± 0.73 ^c	0.79 ± 0.07 ^c	1.30 ± 0.14 ^c	Pyrazine	Caramel, cocoa (μ)	1315–1344	1343 ± 10
30	2,6-dimethyl-pyrazine	0.18 ± 0.01 ^b	0.05 ± 0.01 ^{cd}	0.00 ± 0.00 ^d	0.00 ± 0.00 ^d	1.02 ± 0.07 ^a	0.12 ± 0.01 ^{bc}	Pyrazine	Vegetal, roasted (λ)	1300–1370	1328 ± 11
31	2-acetyl-pyrrole	4.49 ± 0.37 ^a	0.21 ± 0.07 ^c	2.83 ± 0.83 ^b	2.14 ± 0.04 ^b	2.24 ± 0.39 ^b	2.02 ± 0.54 ^b	Pyrrole	Chocolate, hazelnut (μ)	1985	1973 ± 12
32	2-methyl-butanal	4.25 ± 0.29 ^a	4.24 ± 0.36 ^a	2.20 ± 0.15 ^b	3.94 ± 0.51 ^a	1.87 ± 0.08 ^b	2.37 ± 0.19 ^b	Aldehyde	Malty and chocolate (μ)	910	914 ± 8
33	2-methylpropanal	1.28 ± 0.19 ^a	1.18 ± 0.18 ^{ab}	0.51 ± 0.04 ^c	0.92 ± 0.17 ^b	0.48 ± 0.04 ^c	0.55 ± 0.05 ^c	Aldehyde	Chocolate, cocoa, roasted (λ)	817	819 ± 9
34	3,5-dimethyl-2-ethyl-pyrazine	0.01 ± 0.00 ^b	0.06 ± 0.01 ^b	0.00 ± 0.00 ^b	0.00 ± 0.00 ^b	0.54 ± 0.09 ^a	0.08 ± 0.00 ^b	Pyrazine	Earthy, potato-chip (ρ)	1464	1445–1493
35	3-methyl-butanal	3.33 ± 0.32 ^a	2.07 ± 0.34 ^{bc}	1.38 ± 0.12 ^{bcd}	2.10 ± 0.37 ^b	1.06 ± 0.07 ^d	1.27 ± 0.36 ^{cd}	Aldehyde	Malty, chocolate (θ)	912	918 ± 7
36	benzaldehyde	38.51 ± 2.20 ^a	16.44 ± 5.19 ^b	19.14 ± 2.20 ^b	32.01 ± 6.16 ^a	5.41 ± 1.38 ^c	13.53 ± 4.25 ^{bc}	Aldehyde	Sweet, almond, cherry (θ)	1516	1520 ± 14
	Nutty/chocolate	292.79 ± 64.03^a	55.7 ± 9.11^c	161.83 ± 36.05^b	163.27 ± 35.62^b	71.65 ± 13.57^c	61.15 ± 9.34^c				
37	2,3-butanedione	17.39 ± 0.66 ^{bc}	22.81 ± 3.03 ^a	15.46 ± 1.52 ^c	16.52 ± 0.41 ^c	20.92 ± 0.76 ^{ab}	14.37 ± 0.82 ^c	Ketone	Buttery, creamy (α)	975	979 ± 10
38	acetoïn	8.91 ± 0.367 ^{bc}	52.41 ± 9.79 ^a	15.90 ± 2.54 ^b	14.89 ± 0.93 ^c	5.471 ± 0.30 ^{bc}	3.20 ± 0.74 ^c	Ketone	Buttery, sour milk, caramel (μ)	1250	1284 ± 12
39	γ-butyrolactone	1.57 ± 0.09 ^b	3.01 ± 0.82 ^a	3.91 ± 0.75 ^a	1.65 ± 0.14 ^b	1.29 ± 0.05 ^b	1.48 ± 0.38 ^b	Lactone	Sweet, aromatic, creamy (μ)	1618	1632 ± 15
	Creamy/buttery	27.87 ± 7.91^b	78.23 ± 24.86^a	35.27 ± 6.79^b	33.06 ± 8.15^b	27.68 ± 10.33^b	19.05 ± 6.99^c				
40	2-methoxy-phenol	0.56 ± 0.02 ^a	0.66 ± 0.19 ^a	0.17 ± 0.00 ^{bc}	0.26 ± 0.03 ^b	0.28 ± 0.06 ^b	0.00 ± 0.00 ^c	Alcohol	Smoky (ζ)	1848	1861 ± 13
41	3-methylbutanoic acid	42.59 ± 2.49 ^a	24.49 ± 0.64 ^c	20.32 ± 5.52 ^c	45.38 ± 2.40 ^a	38.59 ± 3.91 ^{ab}	28.41 ± 7.83 ^{bc}	Acid	Sweaty (σ)	1676	1666 ± 11
42	acetic acid	528.71 ± 28.24 ^b	797.59 ± 164.29 ^a	525.34 ± 72.01 ^b	498.32 ± 20.00 ^b	417.22 ± 45.15 ^b	348.83 ± 51.19 ^b	Acid	Sharp, pungent, sour (φ)	1452	1449 ± 13
43	ethanol	14.88 ± 1.19 ^a	11.23 ± 1.76 ^b	8.91 ± 0.76 ^b	3.49 ± 0.10 ^c	9.76 ± 0.37 ^b	4.52 ± 0.68 ^c	Alcohol	Alcoholic (α)	929	932 ± 8
	Undesirable	586.74 ± 255.28^b	833.97 ± 392.85^a	554.74 ± 257.90^b	547.45 ± 241.84^b	465.85 ± 201.16^{bc}	381.76 ± 169.38^c				
44	1,2-propanediol, diacetate	2.41 ± 0.11 ^a	2.90 ± 0.82 ^a	0.92 ± 0.17 ^c	2.12 ± 0.27 ^{ab}	2.07 ± 0.41 ^{ab}	1.25 ± 0.14 ^{bc}	Ester	–	–	1525 ± 19
45	2,3-butanediol diacetate	3.09 ± 0.13 ^a	3.05 ± 0.46 ^a	3.18 ± 0.14 ^a	1.95 ± 0.58 ^b	0.80 ± 0.11 ^c	0.51 ± 0.02 ^c	Ester	–	–	1498 ± 15
46	2-ethyl-5-methyl-furan	0.94 ± 0.05 ^a	1.10 ± 0.12 ^a	0.31 ± 0.04 ^c	0.34 ± 0.03 ^c	0.93 ± 0.08 ^a	0.70 ± 0.05 ^b	Furan	Fresh, grassy, burnt (π)	–	1015 ± 3
47	acetaldehyde	0.55 ± 0.03 ^a	0.28 ± 0.08 ^{bc}	0.44 ± 0.09 ^{ab}	0.29 ± 0.02 ^{bc}	0.23 ± 0.05 ^{cd}	0.10 ± 0.00 ^d	Aldehyde	Pungent, ethereal, aldehydic, fruity (π)	–	702 ± 12
48	dimethyl sulfide	0.04 ± 0.01 ^b	0.37 ± 0.10 ^a	0.06 ± 0.01 ^b	0.05 ± 0.01 ^b	0.319 ± 0.07 ^a	0.06 ± 0.01 ^b	Organo-sulfur	Sulfur, cabbage (λ)	716	754 ± 9
	Others	7.03 ± 1.06^a	7.70 ± 0.44^a	4.91 ± 0.19^b	4.75 ± 0.27^{bc}	4.34 ± 0.17^c	2.62 ± 0.049^d				

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Table 2 (continued)

N°	Volatile compounds	SCA12 × ICS40	SNK109 × T79/467	ICS40 × UPA134	ICS40 × SCA12	SNK16 × T60/887	SNK10 × IMC67	Chemical group	Odor description	KI lit.	KI cal.
Grand total		1670.21 ± 296.64 ^a	1575.65 ± 340.37 ^a	1483.23 ± 292.11 ^a	1516.57 ± 308.80 ^a	924.34 ± 196.81 ^b	793.22 ± 166.66 ^b				

Values with the same letter in the same line are not significantly different ($P < 0.05$, Tukey HSD). Values presented are mean values followed by the Standard deviation (SD). KI (lit.): Kovats Retention Index (littérature); KI (exp.): Kovats Retention Index (littérature) (α) Rottiers et al. (2019), (β) Kadow et al. (2013), (γ) Mosciano, Gerard P&F 23, N° 5, 49, (1998), (δ) Mosciano, Gerard P&F 24, N° 2, 49, (1999), (ε) Mosiano, Gerard P&F 25, N°. 4, 71, (2000), (θ) Bastos et al. (2019), (λ) Deuscher et al. (2020), (μ) Utrilla-Vázquez et al. (2020), (π) Thegoodscentscompany.com, (ρ) Meersman et al. (2015), (σ) Assi-;Clair et al. (2019), (q) Perotti et al. (2020) (ω) Tuentier et al. (2020), (ζ) Frauendorfer and Schieberle (2008)

and 2-heptanol and its ester. The SNK109 × T79/467 hybrid was dominated by isoamyl acetate and ethyl acetate. The SNK hybrids and precisely SNK6 × T60/887 and SNK10 × IMC67 were characterized by high contents of 2-heptanol and its ester. Finally, we obtained high percentages of 2-pentanol, butyl acetate, ethyl acetate and isoamyl acetate in ICS40 hybrids. Remarkably, fruity volatiles with high content in all hybrids were esters and secondary alcohols. Many of these compounds were found by Colonges et al. (2022) as in relation with Ecuadorian genotypes. But our results were partly different from those obtained by Rottiers et al. (2019) who identified methyl ketones and their secondary alcohols as key odorants in fruity volatiles. The participation of esters and secondary alcohols in aroma compounds is well known. In fact, Pino, Ceballos, and Quijano (2010) found these compounds among cacao genotypes typical for Columbian plantations. Schwab, Davidovich-Rikanati, and Lewinsohn (2008) and Bastos et al. (2019) defined secondary alcohols as aroma active compounds and highlighted that 2-pentanol and 2-heptanol are important components of passion for fruit flavour.

Specifically, 2,3-butanediol discriminated SCA12 × ICS40 from other hybrids and 2-nonanol and 2-nonanol were specific to fermented dried beans from local Trinitario hybrids (SNK109 × T79/467, SNK6 × T60/887 and SNK10 × IMC67). Cocoa volatiles were highlighted to be an important tool capable of discriminating cocoa genotypes. In fact, Kadow et al. (2013) found that 2-pentanol was specific to SCA6 cocoa and 2-heptanol specific to EET62.

According to several studies, the secondary alcohols and alkyl ketones identified as fruity volatiles in our two matrices are assumed to be fatty acid-derived volatiles (Kadow et al., 2013; Schwab et al., 2008). Esters are generally known as one of the most important contributors to the fruity and floral flavor notes of foods, derived from esterification of alcohols (Jeanjean, 1995, p. 200; Rottiers et al., 2019). They are well represented in fruit aromas and are the second most important group of volatile compounds after pyrazines.

In roasted nibs, the content of total fruity volatiles dropped. This observation was common in all hybrids, but the reduction seemed hybrid dependent. As observed in fermented dried beans, the hybrids ICS40 × SCA12 and SNK10 × IMC67 showed the highest ($416.17 \pm 82.90 \mu\text{g/g}$) and the lowest ($184.53 \pm 23.44 \mu\text{g/g}$) contents of fruity volatiles respectively. The decrease was largely affected by the content of methyl acetate (Table 3). A drastic reduction of volatile compounds from fermented dried beans to chocolates was observed by Tuentier et al. (2020). Due to a drastic reduction in these compounds, some authors only detect them as traces in cocoa by-products (Bonvehi, 2005; Counet, Callemien, Ouwerx, & Collin, 2002).

3.2.2. Floral volatiles

Floral volatiles were represented by terpenes (4), esters (3) and alcohols (2), aldehydes (1) and ketones (1). Harvested under the same conditions, the SNK109 × T79/467 fermented dried beans recorded the highest content of floral aroma volatiles ($97.4 \pm 14.77 \mu\text{g/g}$ dry powder). This hybrid was followed by SCA12 × ICS40 fermented dried beans. The lowest content of floral volatiles was obtained with SNK16 × T60/887 (Table 3). Remarkably, the acetophenone content was specific

to SNK hybrids. Its content represented up to 38.82% in SNK10 × IMC67 fermented dried beans. The relative abundance of acetophenone and its increase in roasted beans was described as conferring floral notes and a sweet flavor to fermented dried and especially roasted beans or chocolate (Bastos et al., 2019). 2-phenyl ethanol from ICS40 × UPA134 showed the highest percentage (up to 49%) of the representativity of a volatile with floral notes. Concretely, 2-phenylethanol and benzyl alcohol were identified as being responsible for odor production with desirable notes (Gálvez, Loiseau, Paredes, Barel, & Guiraud, 2007). Furthermore, authors noted that the high alcohol concentrations confer floral and sweet notes to cocoa fermented dried beans (Aculey et al., 2010). Our results were like those of Liu et al. (2017) who found high content of 2-phenyl ethanol in Cameroonian Forastero. Methylbenzene acetate discriminated hybrids according to their traditional groups. This compound grouped SNK (low content), SCA12 (high content) and ICS40 (moderate content) hybrids in one subgroup. The synthesis of esters by yeasts from alcohols during anaerobic respiration is documented (Rojas et al., 2002). A particularity of floral compounds was observed in SCA12 × ICS40 hybrid. In fact, this hybrid showed important and specific concentration of terpenes (β-myrcene, *trans*-β-ocimene, linalool). Linalool and its derivatives are often described as having a floral aroma and are considered key aroma components found in high concentrations in “noble flavor” cocoas such as Criollo and Arriba (Owusu, Petersen, & Heimdal, 2012). The concentration of linalool was up to $0.5 \mu\text{g/g}$ in SNK10 × IMC67 beans and SCA12 × ICS40 hybrids more precisely. It is well known that cocoa beans with linalool concentrations up to $0.5 \mu\text{g/kg}$ are classified as fine cocoa (Ziegler, 1990). β-myrcene and *cis/trans*-β-ocimene were detected in SCA 6 cotyledons and were associated to a fine chocolate aroma (Kadow et al., 2013). According to Rottiers et al. (2019), linalool, *cis*-linalool oxide, β-myrcene and ocimene are derived from the terpenoid pathway and Colonges et al. (2021) showed a correlation between genotype and these aroma compounds.

An increase of total floral volatiles was observed in roasted nibs compared to fermented dried beans in all hybrids. This increase concerned mainly terpenes and alcohols. The predominant compounds obtained in fermented dried beans of each hybrid were the same in roasted nibs.

3.2.3. Chocolate/nutty volatiles

Chocolate and nutty-like volatiles (10 compounds) were dominated by pyrazines (5), aldehydes (4) and pyrrole (1). The high concentration of chocolate and nutty-like volatiles was obtained in SCA12 × ICS40 fermented dried cocoa beans followed by those from ICS40 hybrids (Table 2). Among aldehydes, benzaldehyde (almond) was the predominant compound found as nutty volatile. It was reported that this aldehyde together with 2-methylbutanal and 3-methylbutanal are produced during roasting via Maillard reaction (Counet et al., 2002; Diab et al., 2014) but also by lactic acid bacteria during fermentation from phenylalanine, isoleucine, and leucine respectively (Bonnamme et al., 2004; Jinap et al., 1994).

The presence of pyrazines in fermented dried cocoa beans was considered as an indicator of a good fermentation process and predicted the quality of beans (Jinap et al., 1994). Tetramethyl and trimethyl

Table 3Average concentrations (mean \pm SD) of volatile compounds ($\mu\text{g/g}$ nibs 1-butanol equivalents) identified in roasted cocoa nibs by HS–SPME–GC–MS ($n = 3$) and Kovats Retention Index.

N°	Volatile compounds	SCA12 \times ICS40	SNK109 \times T79/467	ICS40 \times UPA134	ICS40 \times SCA12	SNK16 \times T60/887	SNK10 \times IMC67	RT (min)	KI (lit.)	KI (exp.)
1	2-nonanol	0.17 \pm 0.05 ^c	1.21 \pm 0.14 ^a	0.29 \pm 0.05 ^d	0.24 \pm 0.04 ^{de}	0.54 \pm 0.03 ^c	0.84 \pm 0.09 ^b	37.56	–	1521 \pm 11
2	2,3-butanediol	0.63 \pm 0.03 ^a	0.03 \pm 0.00 ^c	0.00 \pm 0.00 ^c	0.00 \pm 0.05 ^b	0.03 \pm 0.00 ^c	0.00 \pm 0.00 ^c	38.26	1492–1582	1533 \pm 14
3	2-heptanol	26.32 \pm 2.39 ^c	30.97 \pm 2.80 ^c	6.25 \pm 0.58 ^d	7.33 \pm 0.93 ^d	53.04 \pm 3.55 ^a	42.77 \pm 0.75 ^b	25.16	1326	1320 \pm 9
4	2-heptanol acetate	19.62 \pm 1.74 ^{ab}	5.14 \pm 0.73 ^c	2.77 \pm 0.27 ^c	4.56 \pm 0.59 ^c	21.41 \pm 3.33 ^a	15.75 \pm 0.32 ^b	21.22	1250	1255 \pm 3
5	2-heptanone	0.42 \pm 0.07 ^{bc}	0.03 \pm 0.00 ^d	0.64 \pm 0.06 ^a	0.86 \pm 0.21 ^{ab}	0.45 \pm 0.08 ^{bc}	0.22 \pm 0.00 ^{cd}	15.57	1181	1182 \pm 8
6	2-nonanone	5.42 \pm 0.32 ^{bc}	19.71 \pm 3.84 ^a	3.04 \pm 0.76 ^c	2.93 \pm 0.52 ^c	9.25 \pm 0.39 ^b	9.21 \pm 0.41 ^b	28.90	1389	1390 \pm 7
7	2-pentanol	8.44 \pm 0.21 ^c	19.48 \pm 1.31 ^a	18.71 \pm 0.85 ^b	14.12 \pm 0.63 ^a	8.34 \pm 0.48 ^c	6.89 \pm 0.28 ^c	13.12	1122	1119 \pm 10
8	2-pentanol acetate	5.70 \pm 0.37 ^b	4.66 \pm 0.49 ^{bc}	10.72 \pm 0.66 ^a	9.58 \pm 0.43 ^a	5.18 \pm 0.14 ^b	3.59 \pm 0.21 ^c	10.33	1050	1069 \pm 5
9	butyl acetate	11.93 \pm 1.91 ^a	9.80 \pm 2.50 ^{ab}	11.35 \pm 1.44 ^a	12.04 \pm 1.36 ^a	4.58 \pm 1.61 ^c	5.86 \pm 1.02 ^{bc}	10.26	1075	1074 \pm 8
10	ethyl acetate	7.40 \pm 1.53 ^c	38.78 \pm 4.18 ^a	13.94 \pm 3.40 ^{bc}	16.30 \pm 5.11 ^b	9.07 \pm 1.33 ^{bc}	8.94 \pm 1.76 ^{bc}	4.55	872	888 \pm 8
11	ethyl isopentanoate	0.19 \pm 0.06 ^a	0.20 \pm 0.05 ^a	0.28 \pm 0.09 ^a	0.16 \pm 0.05 ^a	0.14 \pm 0.01 ^a	0.24 \pm 0.01 ^a	10.09	1072	1068 \pm 8
12	isoamyl acetate	8.96 \pm 0.97 ^d	36.21 \pm 2.68 ^a	20.20 \pm 2.50 ^c	30.30 \pm 2.67 ^b	6.02 \pm 1.12 ^d	5.63 \pm 0.13 ^d	12.67	1118	1122 \pm 7
13	isoamyl alcohol	0.52 \pm 0.09 ^{bc}	6.05 \pm 0.85 ^a	1.21 \pm 0.08 ^{bc}	1.46 \pm 0.29 ^b	0.32 \pm 0.08 ^c	0.44 \pm 0.03 ^{bc}	17.97	1214	1209 \pm 9
14	isobutyl acetate	0.06 \pm 0.02 ^c	0.10 \pm 0.01 ^b	0.03 \pm 0.00 ^d	0.04 \pm 0.00 ^{cd}	0.16 \pm 0.01 ^a	0.07 \pm 0.00 ^{bc}	8.45	1008	1012 \pm 8
15	methyl acetate	180.64 \pm 50.45 ^{ab}	226.19 \pm 52.50 ^{ab}	323.26 \pm 83.84 ^a	316.25 \pm 56.31 ^a	86.17 \pm 5.60 ^b	84.92 \pm 28.37 ^b	3.78	813	828 \pm 6
	Fruity notes	276.42 \pm 45.53^b	398.56 \pm 56.88^a	412.69 \pm 82.11^a	416.41 \pm 80.24^a	204.70 \pm 24.23^c	185.37 \pm 22.81^c			
16	2-phenethyl acetate	5.38 \pm 1.39 ^{bc}	40.52 \pm 6.82 ^a	11.08 \pm 3.50 ^{bc}	10.17 \pm 2.66 ^b	1.55 \pm 0.32 ^c	1.72 \pm 0.22 ^c	53.14	1810	1813 \pm 15
17	2-phenyl ethanal	3.10 \pm 0.26 ^{abc}	3.81 \pm 1.21 ^{ab}	2.92 \pm 0.80 ^{abc}	4.35 \pm 1.50 ^a	1.41 \pm 0.29 ^c	1.57 \pm 0.17 ^{bc}	43.25	1634	1640 \pm 13
18	2-phenylethanol	9.13 \pm 1.78 ^b	65.87 \pm 19.24 ^a	20.83 \pm 3.08 ^b	20.32 \pm 6.67 ^b	3.66 \pm 1.21 ^b	4.44 \pm 0.43 ^b	57.39	1891	1906 \pm 15
19	acetophenone	7.39 \pm 0.44 ^b	13.23 \pm 3.27 ^a	4.97 \pm 1.07 ^b	6.71 \pm 1.67 ^b	8.87 \pm 2.56 ^{ab}	7.40 \pm 0.51 ^b	43.74	1642	1647 \pm 13
20	benzyl alcohol	0.70 \pm 0.08 ^b	1.46 \pm 0.25 ^a	0.40 \pm 0.06 ^{bc}	0.66 \pm 0.18 ^b	0.15 \pm 0.05 ^c	0.28 \pm 0.01 ^c	56.20	1872	1870 \pm 14
21	cis/trans- β -ocimene	18.40 \pm 1.26 ^a	0.00 \pm 0.00 ^b	0.06 \pm 0.01 ^b	0.29 \pm 0.05 ^b	0.14 \pm 0.02 ^b	0.51 \pm 0.03 ^b	19.20	1241	1250 \pm 6
22	cis-linalool oxide	1.69 \pm 0.17 ^a	0.87 \pm 0.12 ^{bc}	1.17 \pm 0.13 ^b	1.65 \pm 0.31 ^a	0.60 \pm 0.04 ^c	0.44 \pm 0.01 ^c	34.07	1423	1444 \pm 19
23	ethyl phenyl acetate	0.79 \pm 0.21 ^b	3.34 \pm 0.93 ^a	1.27 \pm 0.29 ^b	0.49 \pm 0.11 ^b	0.24 \pm 0.03 ^b	0.23 \pm 0.03 ^b	51.61	–	1783 \pm 10
24	linalool	3.81 \pm 0.73 ^a	0.09 \pm 0.01 ^b	0.48 \pm 0.11 ^b	0.46 \pm 0.09 ^b	0.38 \pm 0.08 ^b	0.60 \pm 0.09 ^b	39.01	1537	1547 \pm 7
25	methyl-phenyl acetate	10.33 \pm 0.86 ^a	1.02 \pm 0.25 ^d	4.29 \pm 1.00 ^{bc}	6.96 \pm 1.96 ^b	1.46 \pm 0.58 ^d	2.21 \pm 0.17 ^{cd}	50.12	1747	1750 \pm 7
26	β -myrcene	20.17 \pm 2.49 ^a	0.11 \pm 0.00 ^b	0.67 \pm 0.08 ^b	0.72 \pm 0.14 ^b	0.24 \pm 0.04 ^b	0.85 \pm 0.04 ^b	14.86	1159	1161 \pm 7
	Floral notes	80.89 \pm 6.72^b	130.33 \pm 21.54^a	48.14 \pm 6.33^c	52.78 \pm 6.16^c	18.7 \pm 2.59^d	20.25 \pm 2.21^d			
27	2,3,5,6-tetramethyl-pyrazine	205.80 \pm 13.40 ^a	33.33 \pm 6.51 ^c	104.28 \pm 17.05 ^b	98.20 \pm 5.09 ^b	46.16 \pm 11.01 ^c	23.01 \pm 0.53 ^c	34.19	1489	1469 \pm 7
28	2,3,5-trimethyl-pyrazine	28.39 \pm 2.38 ^a	21.96 \pm 3.44 ^{ab}	15.27 \pm 2.48 ^{bcd}	8.28 \pm 1.67 ^d	16.05 \pm 1.70 ^{bc}	11.00 \pm 0.32 ^{cd}	29.82	1408	1402 \pm 8
29	2,3-dimethyl-pyrazine	5.40 \pm 0.58 ^a	5.22 \pm 0.97 ^a	2.43 \pm 0.15 ^b	1.31 \pm 0.16 ^b	1.09 \pm 0.31 ^b	1.70 \pm 0.14 ^b	26.01	1315–1344	1343 \pm 10
30	2,6-dimethyl-pyrazine	0.90 \pm 0.03 ^c	6.78 \pm 0.78 ^a	0.15 \pm 0.03 ^c	0.06 \pm 0.01 ^c	1.17 \pm 0.56 ^c	2.73 \pm 0.25 ^b	24.55	1300–1370	1328 \pm 11
31	2-acetyl-pyrrole	6.18 \pm 0.42 ^b	9.84 \pm 2.34 ^a	3.91 \pm 0.47 ^b	4.45 \pm 1.16 ^b	3.91 \pm 1.12 ^b	3.67 \pm 0.11 ^b	58.84	1985	1973 \pm 12
32	2-methyl-butanol	3.55 \pm 0.37 ^c	6.74 \pm 0.84 ^a	3.43 \pm 0.47 ^c	3.84 \pm 0.14 ^{bc}	2.08 \pm 0.34 ^d	4.93 \pm 0.41 ^b	5.00	910	914 \pm 8
33	2-methyl-propanal	1.03 \pm 0.20 ^{ab}	1.37 \pm 0.09 ^a	0.73 \pm 0.19 ^{bc}	0.84 \pm 0.10 ^{bc}	0.53 \pm 0.13 ^c	1.04 \pm 0.13 ^{ab}	3.62	817	819 \pm 9
34	3,5-dimethyl-2-ethyl-pyrazine	0.14 \pm 0.01 ^b	2.53 \pm 0.55 ^a	0.02 \pm 0.00 ^b	0.04 \pm 0.00 ^b	0.18 \pm 0.04 ^b	0.39 \pm 0.02 ^b	32.37	1464	1445–1493
35	3-methyl-butanol	3.34 \pm 0.13 ^a	2.63 \pm 0.36 ^b	1.58 \pm 0.12 ^{cd}	2.12 \pm 0.28 ^{bc}	1.01 \pm 0.21 ^d	1.84 \pm 0.06 ^c	5.10	912	918 \pm 7
36	benzaldehyde	41.19 \pm 2.68 ^a	19.75 \pm 4.72 ^b	20.71 \pm 3.33 ^b	43.76 \pm 13.18 ^a	6.97 \pm 2.06 ^b	12.14 \pm 0.16 ^b	36.28	1516	1520 \pm 14
	Nutty/chocolate	295.88 \pm 63.41^a	110.15 \pm 10.54^{bc}	152.51 \pm 32.05^b	162.9 \pm 31.64^b	79.15 \pm 14.27^c	62.45 \pm 7.15^c			
37	2,3-butanedione	13.89 \pm 0.25 ^{ab}	12.60 \pm 0.33 ^b	15.06 \pm 1.62 ^{ab}	14.07 \pm 2.01 ^{ab}	16.85 \pm 0.84 ^a	12.07 \pm 1.07 ^b	6.59	975	979 \pm 10
38	acetoin	21.17 \pm 0.72 ^{bc}	66.74 \pm 11.05 ^a	31.59 \pm 6.06 ^b	21.58 \pm 0.73 ^{bc}	13.57 \pm 1.95 ^{cd}	6.69 \pm 0.47 ^d	22.01	1250	1284 \pm 12
39	γ -butyrolactone	1.85 \pm 0.17 ^b	4.00 \pm 0.55 ^a	3.80 \pm 0.53 ^a	2.24 \pm 0.35 ^b	1.40 \pm 0.30 ^b	1.40 \pm 0.07 ^b	42.18	1618	1632 \pm 15
	Cream/buttery	36.91 \pm 9.75^{bc}	83.34 \pm 34.01^a	50.45 \pm 13.87^b	37.87 \pm 5.92^{bc}	31.82 \pm 8.14^{bc}	20.16 \pm 5.33^c			
40	2-methoxy-phenol	0.62 \pm 0.12 ^a	0.53 \pm 0.13 ^a	0.17 \pm 0.02 ^b	0.22 \pm 0.06 ^b	0.25 \pm 0.05 ^b	0.14 \pm 0.01 ^b	55.33	1848	1861 \pm 13
41	3-methyl-butanoic acid	47.29 \pm 13.26 ^a	24.78 \pm 5.36 ^c	27.86 \pm 7.25 ^c	44.60 \pm 15.39 ^a	36.41 \pm 6.92 ^b	35.13 \pm 0.84 ^b	45.70	1676	1666 \pm 11
42	acetic acid	673.87 \pm 59.76 ^b	915.18 \pm 121.38 ^a	577.09 \pm 73.20 ^{bc}	673.00 \pm 75.91 ^b	487.64 \pm 76.25 ^{cd}	377.69 \pm 11.74 ^d	31.80	1452	1449 \pm 13

(continued on next page)

Table 3 (continued)

N°	Volatile compounds	SCA12 × ICS40	SNK109 × T79/467	ICS40 × UPA134	ICS40 × SCA12	SNK16 × T60/887	SNK10 × IMC67	RT (min)	KI (lit.)	KI (exp.)
43	Ethanol	1.77 ± 0.36 ^d	9.33 ± 0.62 ^a	4.09 ± 0.66 ^c	4.70 ± 1.26 ^{bc}	3.67 ± 0.27 ^{cd}	6.33 ± 0.92 ^b	5.59	929	932 ± 8
	Undesirable notes	723.55 ± 329.37 ^b	949.82 ± 451.92 ^a	609.21 ± 283.45 ^c	722.52 ± 328.85 ^b	527.97 ± 237.65 ^d	419.29 ± 206.59 ^e			
44	1,2-popanediol, diacetate	3.21 ± 0.63 ^{ab}	3.58 ± 1.12 ^a	0.90 ± 0.07 ^c	2.63 ± 0.34 ^{ab}	2.11 ± 0.24 ^{abc}	1.76 ± 0.17 ^{bc}	37.40	–	1525 ± 19
45	2,3-butanediol, diacetate	3.66 ± 0.38 ^a	3.07 ± 0.35 ^a	3.92 ± 0.57 ^a	2.80 ± 0.65 ^a	0.95 ± 0.20 ^b	0.50 ± 0.16 ^b	35.16	–	1498 ± 15
46	2-ethyl-5-methyl-furan	0.90 ± 0.06 ^a	0.89 ± 0.08 ^a	0.34 ± 0.02 ^c	0.36 ± 0.01 ^c	1.03 ± 0.10 ^a	0.55 ± 0.04 ^b	8.47	–	1015 ± 3
47	acetaldehyde	0.62 ± 0.04 ^a	0.34 ± 0.09 ^{bc}	0.44 ± 0.09 ^b	0.38 ± 0.03 ^b	0.20 ± 0.05 ^{cd}	0.13 ± 0.019 ^d	2.96	–	702 ± 12
48	dimethyl sulfide	0.04 ± 0.00 ^b	0.16 ± 0.04 ^a	0.06 ± 0.02 ^b	0.06 ± 0.02 ^b	0.20 ± 0.04 ^a	0.03 ± 0.00 ^b	3.21	716	754 ± 9
	Others	8.43 ± 1.1.63 ^a	8.04 ± 1.60 ^a	5.66 ± 1.58 ^b	6.23 ± 1.34 ^b	4.49 ± 0.78 ^{bc}	2.97 ± 0.68 ^c			
	Grand total	1422.08 ± 267.73 ^b	1680.24 ± 350.00 ^a	1278.66 ± 243.76 ^c	1398.73 ± 282.89 ^b	866.83 ± 201.52 ^c	710.35 ± 161.66 ^c			

Values with the same letter in the same line are not significantly different ($P < 0.05$, Tukey HSD). Values presented are mean values followed by the Standard deviation (SD).

pyrazines recorded a high content in nutty/chocolate flavor. Similar results were reported by Rottiers et al. (2019) and Hashim, Selamat, Muhammad, and Ali (1998). The percentage of tetramethyl pyrazine ranged from 72.21% (ICS40 × UPA134) to 37.74% (SNK10 × IMC67) in fermented dried beans. Pyrazine compounds are common in fermented fried foods. In fact, several studies mentioned the microbial origin of some pyrazines in fermented food as cocoa and soybeans (Besson, Creuly, Gros, & Larroche, 1997; Selamat, Harun, & others, 1994). A part of cocoa/chocolate aroma was attributed to tetramethyl and trimethyl pyrazines which form the so-called basic notes (Sukha, Bharath, Ali, & Umaharan, 2013). Our results highlighted the discrimination of hybrids according to their pyrazine concentrations. Similar results were obtained by Ziegler (2009) who found Ghanaian Forastero richer in pyrazines (6.98 mg/kg) than Mexican Criollo (1.42 mg/kg).

Roasted nibs showed higher content of total nutty/chocolate volatiles in all hybrids except in ICS40 hybrids. The content of pyrazines and aldehydes increased during nibs roasting. These observations were in accordance with literature (Jinap, Wan-Rosli, Russly, & Nurdin, 1998; Owusu et al., 2012). The ratio tetramethyl pyrazine (TMP)/trimethyl pyrazine (TrMP) has been considered as an indicator of good roasting conditions. Apart from fermented dried beans of ICS40 hybrids, values comprised between 1.5 and 2.5 were obtained. Ziegler (2009) considered the interval 1.5 and 2.5 as optimal and values below 1 indicated over roasting. Fermented dried cocoa nibs from ICS40 hybrids seemed to be under-roasted with ratios TMP/TrMP > 5. It is reported that roasting conditions of beans depends on the cocoa genotype, the cocoa material that is used (beans, nibs, or liquor), the type of chocolate to be produced (milk or dark chocolate) and in the desired attributes (Santander-Muñoz, Rodríguez Cortina, Vaillant, & Escobar Parra, 2019). Strecker degradation which takes place simultaneously with Maillard reactions is responsible for an increase in aldehyde concentration. 3-methylbutanal (malty), 2-methylbutanal (chocolate) and benzaldehyde (fine floral index) among major aldehyde compounds from fermentation or Strecker degradation (Afoakwa et al., 2008; Fraendorf & Schieberle, 2008) are crucial aroma compounds that contribute to the chocolate aroma intensity (Van Durme, Ingels, & De Winne, 2016; Saputro, Van de Walle, Hinneh, Van Durme, & Dewettinck, 2018). Indeed, Liu et al. (2017) found high content of these compounds in Cameroonian Forastero cocoas. Another volatile with a concentration increased during roasting is 2-acetylpyrrole. This compound is among those which are generated from Maillard reactions during roasting. According to many authors, pyrroles also contribute to the overall aroma after roasting (Counet et al., 2002; Torres-Moreno, Tarrega, & Blanch, 2020). Although roasting conditions proceeded during this work presented the range of satisfaction, this step led to a

lower increase of aldehydes and pyrazines. This suggests that the roasting step should be adjusted to optimize the increase of aldehydes and pyrazines key odorant compounds in roasted cocoa.

3.2.4. Creamy/buttery volatiles

This group is formed from two ketones (3-hydroxybutanone and 2,3-butanedione) and one lactone (γ -butyrolactone). The 2,3-butanedione showed the highest concentration in all hybrids except in ICS40 × UPA134 and SNK109 × T79/467 in which 3-hydroxybutanone (acetoin) represented 45.08 and 66.99% respectively. Acetoin and 2,3-butanedione were reported to be naturally present in cocoa beans (Ho, Zhao, & Fleet, 2014). Specifically, it was showed that acetoin is mainly produced from pyruvate by alcoholic fermentation and from 2,3-butanediol by yeasts (Pretorius, 2000). In fact, both ketones were reported to be synthesized from pyruvate by yeast metabolism and citric acid by lactic acid bacteria (De Vuyst & Weckx, 2016). Similarly, high content of 2,3-butanedione were obtained with Cameroonian Forastero (Liu et al., 2017). These authors reported the milky flavour of Cameroonian Forastero. The authors associated milky-like volatiles with high content of 2,3-butanedione.

Roasted nibs' concentrations of creamy/buttery volatiles were far higher than those of fermented dried beans. Although the concentration in roasted nibs is higher than that in fermented dried ones, it is necessary to note that this increase is largely dependent on acetoin content. In some hybrids, a relative increase in γ -butyrolactone content was observed during roasting. However, the concentration of 2,3-butanedione decreased during roasting and this observation was generalized in all samples. But the decrease in 2,3-butanedione content was less important compared to the increase in acetoin. The increase in acetoin content was very promising because this volatile has been classified as a technological marker for analytes sensitive to cocoa processing (Magaña et al., 2017; Tran et al., 2016).

3.2.5. Undesirable volatiles

This flavor note was formed by four (4) volatiles among which acetic and 3-methylbutanoic acids, ethanol, and 2-methoxy-phenol. Acetic acid was the predominant compound. Similar observations were reported by several authors (Fraendorf & Schieberle, 2008; Tuentner et al., 2020). Acetic acid is responsible for the intense vinegar-like odor which can affect the overall aroma of cocoa. It is known that acetic acid is a result of acetic acid bacteria metabolism in the aerobic phase occurring during fermentation. Moreover, it is the last product formed from ethanol and it is also detected during the analysis. It is surely produced by many yeasts during the anaerobic phase of fermentation (Ho et al., 2014; Koné et al., 2016). The 3-methylbutanoic acid was the

second most representative volatile beside acetic acid. It is considered as an off-flavor volatile (active odor acid) and may be derived from the amino acid Leucine by aerobic putrefactive bacteria or from isobutyl acetate.

Roasted nibs were associated with an increase of total undesirable volatiles especially acetic and 3-methylbutanoic acids. These results were surprisingly opposed to those of [Frauendorfer and Schieberle \(2008\)](#). In line with our results, [Hinneht et al. \(2020\)](#) also observed an increase of acetic and 3-methylbutanoic acids and explained it by the unlocking effect of the heat treatment in the liberating acids from the matrix effect. The increase of acetic acid content in all hybrids was opposed to the decrease of methyl acetate during roasting. A second hypothesis should be the hydrolysis of methyl acetate into methanol and acetic acid at elevated temperature in presence of strong acids. Finally, a drastic decrease of acetic acid content not during roasting but by nibs' grinding step (data not shown).

3.2.6. Others

The last flavor note was made of compounds whose description was absent or those that did not affect the overall cocoa aroma either in fermented dried beans or in roasted nibs. Butanediol diacetate and 1,2-propanediol diacetate were the predominant of in this category defined by five compounds in the two matrices. The total undefined volatiles moderately increased during roasting.

An overview of different volatiles and specific chemical groups allowed constructing a Venn diagram ([Fig. 2](#)). From this, fruity volatiles (fruity flavors) were determined by three chemical groups of compounds namely ketones, esters, and alcohols. These observations were in accordance with literature ([Álvarez et al., 2012](#); [Aculey et al., 2010](#); [Ducki, Miralles-Garcia, Zumbé, Tornero, & Storey, 2008](#)). Floral volatiles were formed by terpenes, ketones, alcohols, aldehydes, and esters. Floral flavors were specific to terpenes ([Fig. 2](#)) well represented by linalool and β -myrcene. Nutty/chocolate volatiles were obtained by pyrazines, aldehydes, and pyrroles. These were specific to pyrazines and pyrroles. Above, the buttery/creamy volatiles were directed by ketones and lactones but were specific to lactones. Undesirable volatiles were composed of alcohols, acids, and phenols and were specific to acids and

phenols. Organosulfurs and furans were the undefined volatiles in cocoa. Our findings showed that some specific chemical groups can describe the genotypic effect of a given cocoa. It had been shown that terpenes (linalool, β -myrcene) were specific to SCA6 cocoa ([Kadow et al., 2013](#)). Pyrazines (tetramethyl pyrazine) were associated to Mexican Criollo ([Aprotosoie et al., 2016](#)). Consequently, a genotype of cocoa richer in terpenes will show floral flavors same as for genotype rich in pyrazines for chocolate/nutty flavors. As fruity flavors were not specific to a chemical group, authors revealed that these did not have a strong genotypic effect (either male or female parent effects), suggesting that fruity flavors may be affected by local growing environment and postharvest processing contributing to a sense of place or terroir for flavor development in cocoa ([Guittard, 2005](#); [Nesto, 2010](#); [Sukha, Bharath, Ali, Umaharan, 2014](#)). Nevertheless, additional investigations are required. Recent studies ([Colonges et al., 2022](#)) have revealed a susceptible link between genomic data and fruity volatiles in Ecuadorian cocoa.

By linking mainly reducing sugars and total aroma content in roasted beans, fermented dried beans from SCA12 \times ICS40 hybrid with the highest content of fructose and glucose did not present the highest concentration of total volatile compounds in roasted beans. Moreover, the hybrid ICS40 \times SCA12 with a low reducing sugar content compared to ICS40 \times UPA134, presented a high level of total volatiles among the two ICS40 hybrids in roasted beans. The participation of aroma precursors to the volatile compounds of cocoa beans is well known. However, the direct correlation between the amount of aroma precursors (reducing sugars and amino acids) to the total aroma volatile remains a controversy. Many authors found that there is no clear quantitative correlation between the amounts of precursors and the aromatic compounds formed ([Tran et al., 2015](#); [Frauendorfer & Schieberle, 2008](#)) while others claim that Criollo cocoa beans contain high levels of precursors that can produce high levels of pyrazines ([Giacometti, Jolic, & Josic, 2015](#)). In accordance with [Giacometti et al. \(2015\)](#), our findings showed that the hybrid SCA12 \times ICS40 with the most important reducing sugar content presented the highest concentration of pyrazines among hybrids.

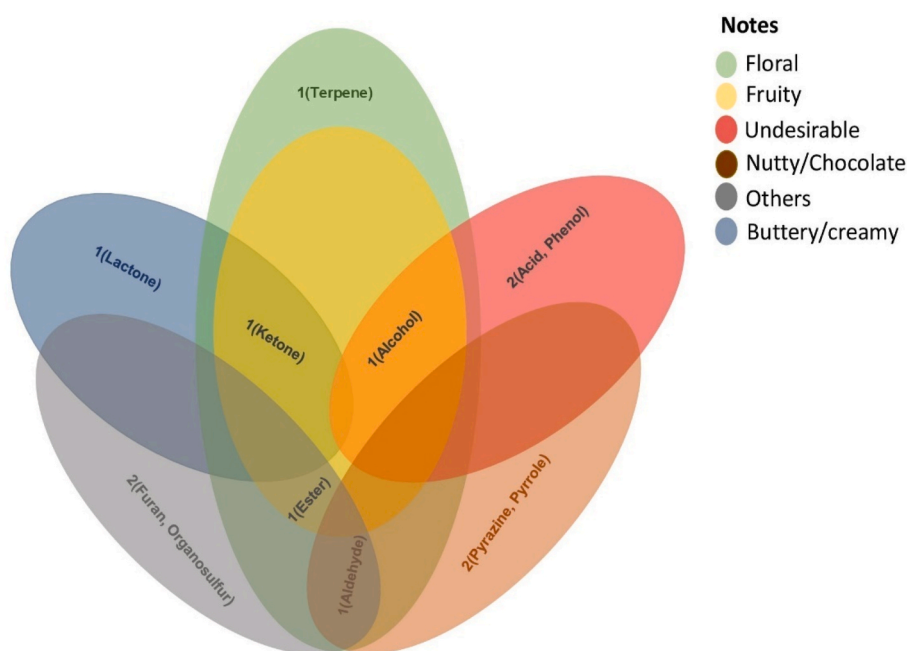


Fig. 2. Venn diagram constructed between aroma volatiles and chemical groups defining each flavor note. Each colour indicates each class of aroma volatiles. Numbers indicate chemical group (s) which (s) is (are) mentioned in bracket. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

3.3. Odor activity values (OAVs) of aroma components in fermented dried beans (FDB) and roasted nibs (RN) of Cameroonian fine cocoas

Several compounds showed high concentrations, but all may not participate strongly in the overall aroma of cocoa beans or roasted cocoa nibs. The participation of a volatile compound into the overall aroma strongly depends on the odor-active value (OAV) of this compound. In accordance with this observation, Calva-Estrada, Utrilla-Vázquez, Vallejo-Cardona, Roblero-Pérez, and Lugo-Cervantes (2020) highlighted that an evaluation of odor Activity values (OAV) is necessary to estimate whether odorants are present in concentrations above their odor thresholds in dark chocolates and whether they play a key role in the authenticity of cocoa. The OAV of a volatile is calculated by dividing the concentration of the volatile aroma by its odor threshold value (OTV) in a specific medium (Van Gemert, 2011). For cocoa and cocoa by-products, the odor threshold values considered are those from volatiles in oil/lipidic media as cocoa contains at least 50% of fats (Belitz, Grosch, & Schieberle, 2009, pp. 340–400). The OTVs of predominant volatiles in lipidic/oil medium are shown in Table 4. It had been found that a volatile with an $OAV \geq 1$ contributes to the aroma as odor-active component to the cocoa/chocolate overall aroma profile (Frauendorfer et Schieberle, 2008; Rottiers et al., 2019).

Acetic acid had the most important OAV in all samples considering the two matrices excepted in fermented dried beans of SNK10 × IMC67 and SNK16 × T60/887 (Table 4). Both concentrations and OAVs increased by roasting. A reduction of volatiles was observed during cocoa processing. Particularly, it was noted that the acid reduction is mainly due to the conching step and the “dilution of the cocoa liquors” by the addition of sugar and cocoa butter (Counet et al., 2002). OAV of acetophenone was more than 1 in SNK hybrids. Kadow et al. (2013) reported the role played by this volatile in enhancing floral character in fine flavor genotypes. With OAVs from 17.35 to 222.18 for 2-phenylethanol and from 7.45 to 295.77 for its ester, these two compounds and precisely 2-phenyl ethanol were recognized as odor-active compounds, adding flowery, honey-like flavor notes (Rottiers et al., 2019). The OAVs

of major aldehydes and pyrazines increased with roasting as these volatiles are known to born or increase during roasting. Also, the content of terpenes increased during roasting. However, the OAVs tendency of major volatiles from fermented dried beans to roasted nibs seemed to be specific to each hybrid (Table 4). The high OAVs obtained from 3-methylbutanal and benzaldehyde in SCA12 × ICS40, SNK109 × T69/467 and ICS40 hybrids might contribute to the chocolate-like and almond-like flavors well described for these two volatile compounds respectively.

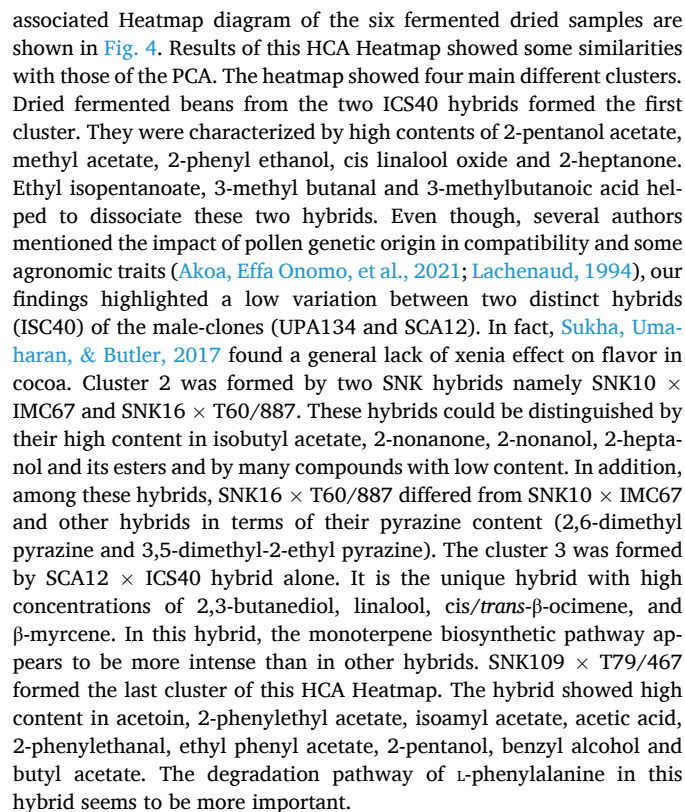
3.4. Principal component analysis (PCA) and hierarchical cluster analysis heatmap (HCA heatmap)

In order to visualize the cocoa hybrids (fermented dried beans or roasted nibs) and to localize volatiles responsible for variations, a PCA was built. It was performed using the content of each volatile obtained after chromatographic (GC-MS) analysis. A biplot of the first two principal components (PC1 = 35.12% and PC2 = 32.44%) is shown in Fig. 3A for fermented dried beans, where a total of 67.56% of the variability is explained. Two hybrids of SNK namely SNK10 × IMC67 and SNK16 × T60/887 were located at the negative axis of F1 and were correlated positively to 2,6-dimethyl-pyrazine, isobutyl acetate, 2-heptanol, acetate and negatively to butyl acetate, methoxy-phenol, ethyl acetate, 2-methyl-propanal, and 2-methyl-butanol. Contrarily, the hybrid SCA12 × ICS40 is located at the positive axis of F2 and F1 while the hybrid SNK109 × T79/467 was found in the negative axis of F2. The hybrid SCA12 × ICS40 showed a greater relationship with a lot of compounds and recorded the greatest abundance with terpenes (linalool, β-myrcene, cis-linalool oxide and cis/trans-ocimene), 2,3-butanediol, 2,3,5,6-tetramethyl-pyrazine, 2,3-dimethyl-pyrazine, methyl acetate, methyl phenyl acetate, and aldehydes (acetaldehyde, 3-methylbutanal, and benzaldehyde). Inversely, the hybrid SNK109 × T79/467 showed its greater correlation with alcohols (2-phenylethanol, isoamyl alcohol, benzyl alcohol, and 2-pentanol), esters (2-phenylethyl acetate, ethyl phenyl acetate and isoamyl acetate) and acetoin, acetic acid, and 2-phenyl ethanol. The two hybrids with the same mother clone ICS40

Table 4
Odor Activity Values (OAVs) of some abundant volatile compounds in fermented dried beans (DFB) and roasted nibs (RN).

Volatile compounds	OTV*10 ⁻³	SCA12 × ICS40		SNK109 × T79/467		ICS40 × UPA134 OAV		ICS40 × SCA12		SNK16 × T60/887		SNK10 × IMC67	
	(µg/g)	FDB	RN	FDB	RN	FDB	RN	FDB	RN	FDB	RN	FDB	RN
Fruity													
2-heptanol	263	91.56	100.08	129.70	117.76	24.33	23.76	27.11	27.87	195.97	201.67	185.51	162.62
ethyl acetate	1429	33.84	5.17	34.38	27.13	17.19	9.75	10.46	11.40	12.37	6.34	12.37	6.64
isoamyl acetate	96	96.15	93.33	526.46	377.19	202.60	210.42	256.04	315.63	56.46	62.71	55.21	58.65
2-nonanol	100	49.83	54.21	97.23	197.11	30.63	30.42	21.21	29.33	87.94	92.52	99.73	92.11
Floral													
2-phenethyl acetate	137	23.65	39.27	184.38	295.77	61.90	80.88	38.54	74.23	8.83	11.31	7.45	12.55
2-phenyl ethanol	211	33.46	43.27	222.18	312.18	120.05	98.72	76.49	96.30	18.10	17.35	26.40	21.04
acetophenone	5629	1.23	1.31	2.26	2.35	1.06	0.88	0.88	1.19	1.52	1.58	1.59	1.31
linalool	37	103.24	102.97	2.97	2.43	11.35	12.97	10.00	12.43	9.46	10.27	16.49	16.22
β-myrcene	9.18	1854.03	2197.17	0.00	11.98	53.38	72.98	70.81	78.43	43.57	26.14	132.90	92.59
Nutty/chocolate													
2,3,5-trimethyl-pyrazine	290	96.21	97.90	11.00	75.72	55.93	52.66	23.93	28.55	49.55	55.34	37.28	37.93
2,3-dimethyl-pyrazine	123	41.87	43.90	6.75	42.44	21.38	19.76	10.24	10.65	6.42	8.86	10.57	13.82
3-methyl-butanal	13	256.15	256.92	159.23	202.31	106.15	121.54	161.54	163.08	81.54	77.69	97.69	141.54
benzaldehyde	60	641.83	686.50	274.00	329.17	319.00	345.17	533.50	729.33	90.17	116.17	225.50	202.33
Buttery/creamy													
2,3-butanedione	4.5	3864.44	3086.67	5068.89	2800.00	3435.56	3346.67	3671.11	3126.67	4648.89	3744.44	3193.33	2682.22
Undesirable													
3-methylbutanoic acid	22	1935.91	2149.55	1113.18	1126.36	923.64	1266.36	2062.73	2027.27	1754.09	1655.00	1291.36	1596.82
acetic acid	124	4263.79	5434.44	6432.18	7380.48	4236.61	4653.95	4018.71	5427.42	3364.68	3932.58	2813.15	3045.88

FDB: Fermented dried beans RN: Roasted nibs OTV: Odor Threshold Values $OAV = \frac{OTV}{\text{Average Concentration}}$.



4. Conclusion

Both biochemical and aromatic compounds helped differentiating cocoa hybrids. Indeed, roasting allowed decreasing sugar content. The differences in terms of volatile compounds among hybrids were quantitative in general and qualitative for just a few compounds. Therefore, 2,3-butanediol, linalool, β -myrcene, *cis/trans*- β -ocimene, 2-nonanone, 2-nonanol, 2-heptanol, methyl acetate, acetophenone helped to discriminate cocoa hybrids according to their native group (Lower Amazonian cocoa, Upper Amazonian cocoa, Trinitario cocoa and local Cameroonian Trinitario cocoa). However, several volatile components (acetoin, 2-phenylethyl acetate, isoamyl acetate, and acetic acid) were specific to a given hybrid irrespective of its native group. The classification of volatiles according to their flavors identified one or two chemical groups specific to some flavor notes. Therefore, floral, chocolate/nutty, buttery/creamy, and undesirable volatiles were specific to terpenes, pyrazines, lactones, and acids/phenols respectively. Roasting (to be optimized) was marked by a general reduction in total volatile compounds in all hybrids. Nevertheless, the decrease in volatiles was mostly observed with fruity volatiles. The content in floral, chocolate/nutty, buttery/creamy, and undesirable volatiles slightly increased during roasting. No real differentiation in terms of sugar content and volatile concentration was observed amongst hybrids with the same mother-clone, suggesting an absence of pollen effect on these compounds. The variation in content of each volatile compound during roasting of cocoa nibs depends on each hybrid, thus suggesting the impact of cocoa genotype on roasting. It is strongly recommended that future studies should lay emphasis on this aspect.

Simon Perrez akoo: Research planning, Writing - original draft, Investigation, Methodology, Formal analysis and software, Writing - review & editing, Visualization., **Pierre Effa Onomo**: Supervision, Research activity planning, Execution, Writing - review., **Renaud Boulanger**: Investigation, Formal analysis, Writing - review & Editing.,

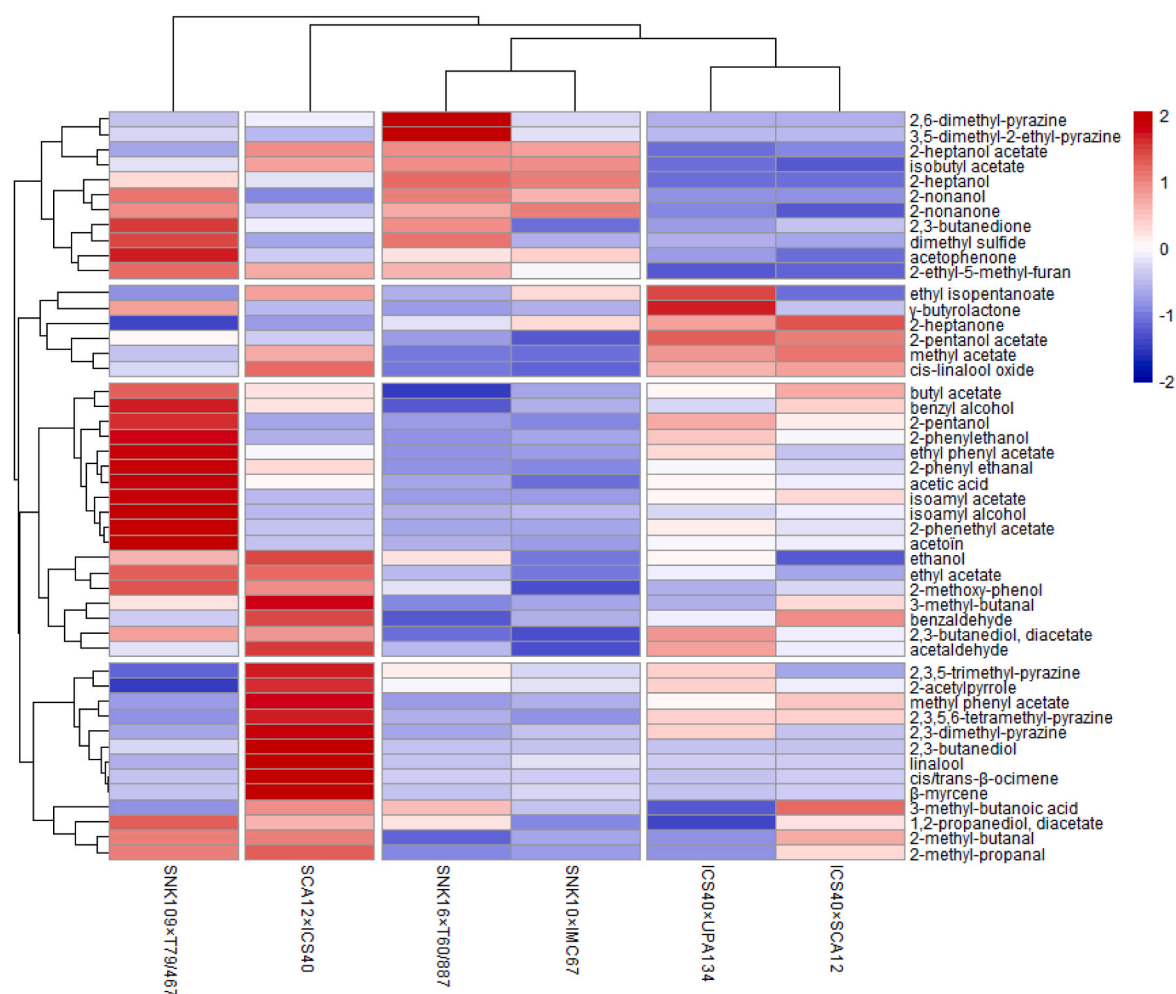


Fig. 4. Dendrogram obtained from HCA (Hierarchical Cluster Analysis) of the 48 identified volatile compounds in fermented dried beans from 6 different cocoa hybrids. The red colour indicates a high abundance while the blue colour indicates a low abundance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Marie-Christine Lahon: Lab supervision., **Martine Louise Ondobo:** Supervision., **Nicolas Niemenak:** Supervision., **Samuel Arsène Ntyam Mendo:** Conceptualization, Software., **Marc Lebrun:** Investigation, Chemical identification assistance., **Pierre-François Djocgoue:** Supervision & Validation.

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Compliance with ethics requirements

This article does not contain any study with human or animal subjects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fbio.2023.102603>.

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