J Food Sci Technol (November 2022) 59(11):4466–4478
https://doi.org/10.1007/s13197-022-05526-w

ORIGINAL ARTICLE

Effect of spontaneous fermentation location on the fingerprint of volatile compound precursors of cocoa and the sensory perceptions of the end-chocolate

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Revised: 28 September 2021 / Accepted: 10 January 2022 / Published online: 20 June 2022

Abstract Cocoa pod-opening delay and bean fermentation promote the organoleptic quality of chocolate. The present research investigated the changes in the volatile fingerprint of cocoa harvested at a traditional plantation. Cocoa beans extracted from 2-days pod-opening delay were simultaneously fermented for 5 days using container and then sun-dried to 7–8% moisture content at five different locations: Akoupé, San Pedro, Soubré, Djekanou and Daloa. The aromatic analysis were done on cocoa using the HS-SPME-GC/MS technique. Professional panelists evaluated the sensory perceptions of the chocolate. The results shows that cocoa fermented in both Daloa and Soubré regions were differentiated by 2,3-butanediol while those processed in other regions presented highest acetoin content. However, fermented cocoa from Soubré region exhibited most amount of 2,3-butanediol, diacetate A whereas 2,3,5,6-tetramethylpyrazine differentiated those from Daloa region. Sensory properties of chocolate were not linked to the aromatic compound precursors profile of beans. The fermentation performed in San Pédro region promote both the generation of more desirable aromatic compounds of cocoa and sensory attributes of the finished chocolate. The fermentation location generates a greater differentiation of the volatile fingerprint of cocoa and the sensory perceptions of the finished chocolate.

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Keywords Cocoa beans · Fermentation location · Aromatic compound precursors · Sensory perceptions · Chocolate

Introduction

Cocoa beans and chocolate are known as luxury foods that provide an astringent taste and typical aroma (Pedan et al. 2017). Chocolate is one of the most consumed foods worldwide mostly because of the unique flavor and sensory characteristics of this product. Cocoa bean is the main raw material for chocolate manufacture. The content and type of flavour compounds in the cocoa beans are certainly the most important indicators of fermented and dried cocoa bean quality (Assa and Yunus 2019) and the most important sensory characteristic of chocolate for the consumer acceptance and preferences (Castro-Alayo et al. 2019). Cocoa beans have intrinsic flavour compounds depending on several factors. Most compound precursors are formated in the cocoa beans during the fermentation and drying process. The fermentation the most crucial stage influencing the aromatic potential of cocoa beans (Mota-Gutierrez et al. 2019) because chocolate flavours are formed mainly from aromatic compound precursors during roasting (Janek et al. 2016). However, the formation of aromatic compound precursors is influenced by several factors, such as type of cocoa bean genotype, soil conditions, the age of the cocoa trees, postharvest treatments, and industrial cocoa processing (Bastos et al. 2019). Currently, several common and main flavor compound families such as aldehydes, ketones, esters, alcohols, acids, pyrazines were identified in cocoa grains and finished chocolate (Koné et al. 2016).

Although Côte d'Ivoire is the world leader in cocoa bean production (Delgado-Ospina et al. 2021), the most relevant research reported that on-farm cocoa bean fermentation is still performed spontaneously (Guéhi et al. 2010). This situation leads to the fermented and dried cocoa beans with poor aromatic quality (Koné et al. 2016). Nowadays, spontaneous fermentation is being abandoned for the benefit of a totally controlled fermentation process which necessitates using microorganisms as a starter culture (Assi-Clair et al. 2019). A large number of relevant research studies reported that the improvement of the aromatic quality of cocoa is possible using specific microorganisms as starter cultures (Assi-Clair et al. 2019). Up today, only a few studies on cocoa fermentation and quality refer to the diversity of fermentative microbiota such as yeasts (Koffi et al. 2017) and the acetic bacteria (Hamdouche et al. 2019). This work aims to evaluate the effects of the fermentation location on the formation of flavour compounds in cocoa beans and the organoleptic quality of the derived chocolate.

Materials and methods

Materials

Fresh cocoa seeds originating from the mature pods of the Ivorian hybrid cultivar, commonly known as "Mercedes" (Amelonado × West African Trinitario) were used in the present work. A total of 2 × 4000 ripe cocoa pods were harvested on one traditional farm during the main harvest season in 2017 and 2018. This 5 hectares plantation (N 06° 30.806′ and W 003° 57.001′) is located in Akoupé region in the south-east of Côte d'Ivoire between 6.38° north latitude and the 3.87° west longitude, about 150 km from Abidjan. The total number of harvested cocoa pods was divided into five 800 pods-batches.

Pod transportation

After one day of the harvesting of the mature and healthy pods from a peasan plantation, the pods batches were transferred by car one day to five cocoa producing regions such as Daloa, Akoupé, San Pédro, Soubre and Djekanou as fermentation locations (Table 1).

Pods opening at the fermentation location

Once at each fermentation location, the cocoa pods were stored for 2 days before disinfecting by soaking in a hydrochloric solution (0.5%) for 15 min and then opened with a wooden billet as bludgeon according to the method of Guéhi et al. (2010). The fresh cocoa beans were extractedmanually without placenta and then sorted in order to discard the rotten beans.

Methods

Cocoa beans fermentation

The fermentation process was carried out in duplicate for 5 days. Germinated, black or diseased cocoa beans were excluded and only healthy beans were fermented. For this, sixty kilograms of beans were divided into two fractions. The beans of each fraction were tipped on the banana leaves. Then they were placed in a plastic box of 40 × 40 × 40 cm^3 mesurements for the fermentation process. The heap of wet cocoa beans was finally covered in the box with more fresh banana leaves to insulate the top of the box before placing the cover according to the methods of Koné et al. (2016). Cocoa beans fermentation treatments were began on the same time at all 5 five fermentation locations. Cocoa beans fermented at Akoupé region were considered as the control in terms of fermentation location The fermenting cocoa beans were turned simultaneously at all fermentation locations after 48 and 96 h.

Table 1 Geographic characteristics of five selective cocoa fermentation regions

Selected cocoa fermentation regions	Geographic location	Distance from Abidjan to the fermenting regions (km)	Altitude (m)	Geographic coordinates
Daloa	Midwest	400	Min. 202 Max. 355	N 06°52′36.085″ W 06°27′5.794″
Akoupé	South East	150	Min. 95 Max. 191	N 06° 23′2728″ W 03°53′23.793″
San Pedro	South West	375	588	N 05° 00′50.947″ W 06°56′24.805″
Soubré	South-center/west	550	131	N 05° 47′6.962″ W 06°35′34.198″
Djékanou	Center	250	185	N 06° 29′2.011″ W 05°05′55.897″

Cocoa beans sampling

Three samples of 500 g of 5 day-fermented cocoa beans were withdrawn from both batches at each fermentation location. For this purpose, all operating workers wore new sterile gloves for the removal of the fermenting cocoa beans from the heap according to the method of Koné et al. (2016). Each fermented bean sample was exposed on the tarpaulin plastic for sun-drying until 7–8% moisture content before chemical analyses were carried out (Koné et al. 2016).

Physical quality traits assessment of cocoa grains

The impact of the fermentation location was evaluated only on the 5 day-fermented cocoa. Bean count was determined following ISO 2451:2017 standard and expressed by the number of whole beans in a 100 g sample. The fermentation degree of the cocoa beans was evaluated with a bean cut test using the Magra bean cutter (Rottiers et al. 2019). The cut test providing data relatde to the sanitary and fermentation quality of cocoa beans was performed using the method previously described (Koné et al. 2021). Then, the cut test score was calculated according to the following previous equation:

$$\text{Cuttestscore} = (10 \times \%\text{Brown}) + \left(5 \times \%\frac{\text{Purple}}{\text{Brown}}\right) + (0 \times \%\text{PurpleandSlaty})$$

Cocoa volatile compound extraction

Fifty grams of dried cocoa grains were manually deshelled and ground into fine powder (0.5 µm) using a Moulinex grinder (John Gordon®, London, United Kingdom) and stored at –20 °C. The volatile compounds were extracted from the cocoa powder (2.5 g) of each fermented and dried cocoa bean sample using the headspace solid-phase micro-extraction technique (HS-SPME) and fibers of 50/30 µm divinylbenzene/carboxene/polydimethylsiloxane (DVB/CAR/PDMS, Supelco, Sigma-Aldrich N.V., Bornem, Belgium) according to the previously described method (Rottiers et al. 2019) but using n-butanol as internal standard. Each volatile compound was identified using three criteria: (i) by comparison of the retention index with the CIRAD aromatic database (Hamdouche et al. 2019), (ii) by matching their mass spectra with those obtained from a commercial database (Wiley275.L, HP product no. G1035 A) and (iii) whenever possible, the identification was confirmed using pure standards of the components (Assi et al. 2019). The peak areas were used for the relative quantification of each volatile compound using the MSD Chemstation software (version E.02.02.1431, Agilent Technologies) as described by Koné et al. (2016). The concentration of each volatile compound was calculated according to the following formula:

$$q_i\left(\mu g.g^{-1}\right) = \frac{25 \times A_i}{A_{but} \times m_e \times W}$$

where

q_i = the concentration of aromatic compound precursor i.
A_i = the area of compound i
A_{but} = the area of butanol (standard)
25 = the content of butanol expressed in mg kg^{-1}
m_e = the mass of sample introduced into the vial in g
W = moisture content of cocoa sample.

Sensory analysis of chocolate

Chocolate was produced from 5 day-fermented cocoa beans sampled from each of the five fermentation locations (Assi et al. 2019). Whole cocoa beans roasted at 160 °C for 30 min (Ramli et al. 2006). For the chocolate sensory perception, twelve judges, six women and six men of professional team frequently trained were asked to smell and taste each chocolate from each cocoa sample against the chocolate manufactured from cocoa beans fermented at Akoupé region considered as control. They found significant differences in organolpetic attributs of chocolate produced from fermented cocoa bean processed at each tested location of fermentation in comparison to the control. The global quality and the intensity of each organoleptic property were evaluated simultaneously using a scale varying from 0 to 10 and a total score for each chocolate sample was assigned.

Statistical analysis

The area of the chromatographic peak of each aromatic compound precursor was calculated using the software (Instrument Data Analysis) and then exported to Excel. The statistical analyses were carried out with the XLSTAT PLS2 (Addinsoft, New York, NY). The sensory perception data were analyzed with Microsoft Excel Program, 2013 (Microsoft Corporation, Redmond, Washington, USA). The testing of the equality of variances was performed with the Fischer's test with a single factor ($p < 0.05$) in order to indicate the significant differences between volatile compound content of fermented and dried cocoa samples, the sensory perception of chocolate produced from tested cocoa as affected by the fermentation location (Koné et al. 2021). Afterwards, the principal component analysis (PCA) was used to visualize the complex data matrix and study the relationship between (i) the generation of volatile aromatic compound precursors as affected by the cocoa fermentation locations, (ii) the profile of the volatile compound precursors and the sensory attributes of chocolates produced from tested beans, (iii) the profiles of the volatile compound precursors and the sensory attributes of chocolates produced from tested beans as affected by fermentation location (Rottiers et al. 2019).

Results and discussion

Physical quality parameters of cocoa bean according to the fermentation location

Table 2 shows the results obtained from the cut test performed on the fermented (120 h), dried beans. All analysed batches contained > 80 beans per 100 g of fermented beans, indicating a good bean count. No statistical difference was found between the bean counts sampled from the five fermentation locations. Low bean counts were due probably to the genotype, the age, growing conditions of the cocoa trees (Kadow et al. 2013) and probaly to the season of production. Low percentages of slaty and moldy beans; and higher percentages (> 5%) of purple beans and defective beans (insect damage, germinated or flat) were detected in all bean samples whatever the fermentation location. The higest percentage of brown beans was detected in the cocoa from San Pédro region. Low percentage of mouldy beans were evaluated in all cocoa batches. This result is in accordance with those previously obtained by Koné et al. (2016). This means that the fermentation have gone very well without fungi growth (Mounjouenpou et al. 2008). This result could be explained by the inhibition of fungi growth by antifungal bacteria and yeasts due to the organic acids producing lactic acid bacteria strains and proteinaceous compounds producing yeast during cocoa fermentation (Ruggirello et al. 2019). In addition, high amounts of alcohol together with low pH, organic acids, elevated temperatures, and microaerophilic conditions inside the fermenting bean mass, such as, are restrictive for the growth of molds (Delgado-Ospina et al. 2021).

Fingerprint of volatile compound precursors in the function of fermentation location of cocoa

Table 3 presentes 34 volatile compound precursors classified in seven chemical families namely: alcohols (6), aldehydes (2), ketones (6), acids (5), esters (10), pyrazines (2) and other compounds (3). This is in accordance with Assi-Clair et al. (2019) who analysed the aromatic compound precursors of Saccharomyces cerevisiae inoculated cocoa bean from Côte d'Ivoire. In addition, the esters had a greater presence in the volatile fingerprint of cocoa samples as found by Valle-Epquin et al. (2020) who analyzed the volatile fingeprint of Criollo cocoa. However, we have detected less volatile compounds than those found in the Criollo cocoa from Amaon region of Peru (Valle-Epquin et al. 2020). Ethanol, 2-Pentanol and 2-Heptanol differentiated unfermented cocoa bean. The highest concentration for unfermented at alcohols could be ascribed to the activities of endogenous invertase (cotyledon and pulp) and toher glycosidases (Hansen et al. 1998) prior pod-opening for extraction of cocoa beans. However, our study highlights more constituent aromatic compound precursors in unfermented cocoa than that of Rottiers et al. (2019). The increase in the production of certain aromatic compound precursors families in cocoa could be due to the microbial metabolism such as yeasts and lactic and acetic acid bacteria, during fermentation (Crafack et al. 2014). The production of these esters can be resulted form yeast

Table 2 Physical quality of cocoa fermented in five fermenting regions of Côte d'Ivoire

Quality parameters	Cocoa beans fermenting sites				
	Daloa	Akoupé	Soubré	Djékanou	San-Pédro
Bean count (number of beans (100 g)$^{-1}$	84 ± 05^a	81 ± 01^a	84 ± 05^a	85 ± 06^a	85 ± 02^a
Bean cut test					
Defective (%)	09 ± 04^a	05 ± 04^a	06 ± 00^a	09 ± 04^a	06 ± 03^a
Slaty (%)	00 ± 00	03 ± 03	00 ± 00	00 ± 00	00 ± 00
Purple (%)	27 ± 03^a	33 ± 13^a	35 ± 10^a	25 ± 03^a	17 ± 07^a
Purple-brown (%)	28 ± 08^a	13 ± 10^a	20 ± 11^a	29 ± 04^a	13 ± 01^a
Brown (%)	40 ± 08^a	49 ± 07^a	42 ± 20^a	41 ± 04^a	64 ± 11^a
Moldy (%)	0	0	0	0	0
Cut test score	540 ± 42^a	555 ± 120^a	520 ± 141^a	555 ± 21^a	705 ± 106^b

Mean ± standard deviation of two repetitions; ab, the values of the same line with different letters show a significant difference (Fisher's test, $p < 0.05$)

Table 3 Main volatile aroma precursor compounds concentration measured in the fermented and dried cocoa beans according to the targeted fermenting sites at Côte d'Ivoire ($N=6$)

Families of compounds	Mains volatile compounds	Odor attributes	Unfermented cocoa beans (μg.g^{-1})	Content of volatile compounds of cocoa grains processed at different fermenting sites				
				Daloa (μg.g^{-1})	Soubré (μg.g^{-1})	Akoupé (μg.g^{-1})	Djékanou (μg.g^{-1})	San-Pédro (μg.g^{-1})
Alcohols	Ethanol	Ethanol-like, sweet pungent	35.54 ± 2.15^{b}	18.06 ± 15.66^{b}	18.07 ± 16.01^{b}	2.74 ± 0.50^{b}	55.55 ± 20.38^{a}	4.74 ± 1.14^{b}
	3-Buten-2-ol, 2-methyl-	Herbal	8.02 ± 0.22^{ab}	7.15 ± 3.12^{ab}	10.47 ± 6.86^{a}	5.44 ± 0.84^{bc}	9.63 ± 2.07^{ab}	1.85 ± 0.61^{c}
	2-Pentanol	Green, Fruity, Sweet, Pungent, Plastic	94.00 ± 3.80^{a}	32.74 ± 11.26^{b}	48.14 ± 21.94^{a}	30.10 ± 7.09^{b}	59.71 ± 5.90^{a}	14.64 ± 5.43^{c}
	2-Heptanol	Fresh lemon, grass herbal, sweet floral, earthy (offflavor)	27.70 ± 4.50^{a}	11.08 ± 0.90^{b}	7.01 ± 4.86 cd	5.40 ± 0.85^{d}	8.75 ± 2.20^{bc}	14.87 ± 1.42^{a}
	2,3-Butanediol	Fruity, creamy, buttery	nd	61.65 ± 33.42^{b}	114.65 ± 70.40^{a}	24.93 ± 6.66^{bc}	27.18 ± 8.30^{bc}	6.18 ± 3.53^{c}
	Phenylethyl Alcohol	Floral rose, dried rose flower, rose water	4.40 ± 0.38 b	14.36 ± 6.61^{b}	08.55 ± 1.85^{b}	12.92 ± 2.60^{b}	30.41 ± 20.71^{a}	11.92 ± 2.38^{b}
Aldehydes	Nonanal	Rose fresh, orange peel	1.70 ± 0.18 b	1.93 ± 0.13^{b}	0.87 ± 0.17cd	1.35 ± 0.76 bc	0.50 ± 0.12 d	4.33 ± 0.94 a
	Benzaldehyde	Sweet, almond, cherry	0.89 ± 0.09^{c}	34.43 ± 9.14^{b}	28.68 ± 9.31 b	34.60 ± 3.06 b	45.38 ± 5.46 a	45.18 ± 10.19 a
Ketones	2,3-Butanedione	Buttery/ creamy	75.00 ± 5.24^{a}	50.00 ± 4.57^{a}	47.20 ± 16.52^{a}	44.48 ± 6.11^{ab}	35.11 ± 10.00^{b}	14.08 ± 7.76^{c}
	2-Heptanone	Fruity, acetone, green banana, with a creamy nuance	11.03 ± 0.85^{bc}	13.44 ± 4.46^{b}	7.84 ± 2.87^{c}	9.56 ± 1.37^{c}	7.40 ± 1.78^{c}	19.07 ± 03.75^{a}
	Acetoin	Buttery, sour milk, caramel	0.42 ± 0.05^{d}	88.70 ± 28.50^{a}	36.75 ± 12.92^{c}	64.23 ± 12.43^{b}	38.15 ± 13.00^{c}	22.36 ± 13.09^{c}
	2-Acetoxy-3-butanone	Sweet, creamy, buttery	nd	29.17 ± 07.87^{a}	9.45 ± 2.96^{c}	19.73 ± 8.17^{b}	6.83 ± 3.08^{c}	3.09 ± 2.15^{c}
	2-Nonanone	Milk, green, fruity	7.71 ± 1.10^{bc}	10.63 ± 0.63^{b}	5.34 ± 1.47 cd	7.15 ± 2.77^{c}	3.52 ± 0.50^{d}	21.33 ± 4.15^{a}
	Acetophenone	Sweet, cherry pit and coumarin	6.08 ± 0.98^{c}	23.10 ± 3.03^{a}	27.28 ± 3.63^{a}	26.00 ± 6.20^{a}	22.56 ± 1.18^{a}	16.95 ± 4.94^{b}
Acids	Acetic acid	Vinegar, peppers, green, sour	21.08 ± 1.57^{e}	2300.00 ± 490.56^{a}	1819.60 ± 479.72^{b}	1924.00 ± 311.67^{ab}	1269.46 ± 246.37^{c}	808.80 ± 219.26^{d}
	Propanoic acid	Pungent, rancid, soy	0.05 ± 0.00^{d}	5.80 ± 0.70^{a}	3.46 ± 0.23^{b}	5.91 ± 0.94^{a}	1.90 ± 0.37^{c}	3.66 ± 1.11^{b}
	Propanoic acid, 2-methyl	Rancid	0.09 ± 0.00^{d}	154.72 ± 41.13^{b}	328.80 ± 75.07^{a}	180.54 ± 55.52^{b}	74.60 ± 10.82^{c}	77.82 ± 37.10^{c}
	Butanoic acid	Cheese	nd	2.26 ± 0.56^{b}	1.18 ± 0.18^{b}	3.82 ± 2.04^{a}	1.51 ± 0.06^{b}	1.17 ± 0.14^{b}
	Butanoic acid, 3-methyl-	Sweaty	0.35 ± 0.06^{d}	62.16 ± 25.88^{b}	39.31 ± 10.62^{c}	111.17 ± 14.70^{a}	27.61 ± 02.47^{c}	102.96 ± 11.53^{a}

Table 3 (continued)

Families of compounds	Mains volatile compounds	Odor attributes	Unfermented cocoa beans (µg.g^{-1})	Content of volatile compounds of cocoa grains processed at different fermenting sites				
				Daloa (µg.g^{-1})	Soubré (µg.g^{-1})	Akoupé (µg.g^{-1})	Djékanou (µg.g^{-1})	San-Pédro (µg.g^{-1})
Esters	Isobutyl acetate	Fruit, apple, banana	0.56 ± 0.08^{c}	52.25 ± 44.51^{a}	24.01 ± 17.00^{b}	24.76 ± 05.55^{b}	32.16 ± 10.35^{ab}	7.11 ± 01.73^{b}
	Butyl acetate	Fruity, floral, banana	2.10 ± 0.15^{d}	23.71 ± 03.16^{a}	20.10 ± 2.67^{ab}	20.53 ± 7.32^{ab}	16.32 ± 4.47^{bc}	13.26 ± 3.12^{c}
	2-Pentanol, acetate	Green, fruity	2.90 ± 0.34 b	46.03 ± 17.88^{a}	48.62 ± 27.92^{a}	38.11 ± 5.70^{a}	38.43 ± 14.03^{a}	16.90 ± 8.93^{b}
	Isoamyl acetate	Sweet fruity banana solvent	2.62 ± 0.33^{d}	124.15 ± 88.64^{a}	61.75 ± 31.07^{bc}	80.44 ± 19.00^{abc}	92.60 ± 13.40^{ab}	36.76 ± 13.33 c
	2-Heptyl, acetate	Woody, citrus, apricot	0.36 ± 0.07^{d}	7.51 ± 1.65^{b}	4.61 ± 3.53^{c}	4.54 ± 0.65^{c}	3.60 ± 1.48^{c}	12.04 ± 3.06^{a}
	2,3-Butanediol, diacetate A	–	0.02 ± 0.00^{c}	93.75 ± 51.73^{b}	160.52 ± 109.81^{a}	36.46 ± 10.14^{bc}	23.76 ± 12.89^{c}	6.36 ± 4.60^{c}
	2,3-Butanediol, diacetate B	–	nd	25.12 ± 10.75^{b}	37.76 ± 16.35^{a}	19.90 ± 7.51^{b}	7.87 ± 2.43^{c}	7.04 ± 2.90^{c}
	Benzeneacetic acid, methyl ester	Honey, sweet	nd^{c}	1.08 ± 0.35^{b}	1.08 ± 0.15^{b}	1.30 ± 0.19^{b}	0.47 ± 0.46^{c}	2.44 ± 0.79^{a}
	Benzeneacetic acid, ethyl ester	Honey, flowery	0.15 ± 0.01^{d}	6.68 ± 2.83^{ab}	6.45 ± 3.04^{ab}	4.64 ± 0.51^{bc}	7.95 ± 0.73^{a}	3.13 ± 1.01^{c}
	2-Phenylethyl Acetate	Honey, flowery	1.68 ± 0.14 c	95.70 ± 53.61^{a}	43.42 ± 17.87^{b}	70.66 ± 17.57^{ab}	85.56 ± 45.62^{a}	34.34 ± 11.16^{b}
Pyrazines	2,3,5-Trimethyl-pyrazine	Cocoa, rusted nuts, peanut	0.12 ± 0.01^{d}	21.51 ± 5.78^{a}	10.25 ± 1.45^{bc}	14.42 ± 1.94^{b}	9.81 ± 3.80^{c}	7.55 ± 3.30^{c}
	2,3,5,6-Tetra-methylpyrazine	Chocolate, cocoa, coffee	0.84 ± 0.11^{e}	492.70 ± 133.93^{a}	141.60 ± 60.30^{c}	281.77 ± 82.06^{b}	120.60 ± 57.70 cd	40.30 ± 30.72^{d}
Terpene	Linalool	Citrus floral, woody, green blueberry	0.28 ± 0.04^{d}	1.57 ± 0.09^{b}	1.70 ± 1.18^{b}	0.81 ± 0.31^{c}	3.31 ± 0.42^{a}	1.16 ± 0.24^{bc}
Furanes	Furfural	Bread, almond, sweet	nd	2.02 ± 0.64^{a}	0.68 ± 0.27^{c}	1.10 ± 0.10^{b}	0.48 ± 0.13^{c}	0.13 ± 0.13^{d}
Sulfur compounds	Dimethyl sulfide	–	1.40 ± 0.04 cd	2.80 ± 0.29^{ab}	3.34 ± 1.26^{a}	2.30 ± 0.21^{b}	2.69 ± 0.60^{ab}	1.22 ± 0.36^{c}

Mean ± standard deviation of six repetitions

Different lowercase letters (a–c), within one row, indicate significant differences ($p < 0.05$) between three batches following one-way ANOVA and post hoc test

[a,b,c,d]the values of the same line with different letters show a significant difference (Fisher's test, $p < 0.05$)

metabolism during the fermentation process, which produces key cocoa aromas such as flowery and honey flavour notes. Our results suggest that not all aromatic compound precursors of bluck cocoa are only due to fermentation but also to the location of fermentation.

Alcohols

All tested cocoa show high production of alcohols whatever the fermentation location. The high production of alcohols could be explained by fermentation of cocoa mucilaginous pulp sugars straing during the post harvest storage of pods (Koné et al. 2016). Cocoa processed in Soubré region exhibited higher concentrations in various alcohols such as ethanol, 2-methyl-3-buten-2-ol, 2-pentanol, 2-heptanol, 2,3-butanediol and 2-phenylethyl alcohol than other cocoas. This could be explained by the involvement of endemic efficient yeast strains in cocoa fermentation. Among these alcoholic compounds, 2,3-butanediol as the most abundant alcohol differentiate cocoa processed in Soubré region with 114.65 ± 70.40 μg.g^{-1} ($p < 0.05$) (Table 3). Valle-Epquín et al. (2020) have reported thet the presence of 2,3-butanediol is desirable for high quality cocoa products. Our results related to 2,3-butanediol; 2-pentanol, 2-heptanol and 2-ethyl-1-hexanol are in agreement with those obtained by Kadow et al. (2013). Bastos et al. (2019) have reported that 2-heptanol is the main cocoa alcoholic volatile compound. Phenylethyl alcohol differentiate cocoa from Djékanou region with significant high content of 30.41 ± 20.71 μg.g^{-1} ($p < 0.05$). Although certain alcohols are benficial for the aromatic cocoa quality due to the formation of various aromatic compounds such as phenyl acetaldehyde and 2-phenylethyl acetate which have the fruity and floral notes (Valle-Epquín et al. 2020; Bastos et al. 2019). The concentration in 2-pentanol detected in cocoa from both Soubré (48.14 ± 21.94 μg.g^{-1}) and Djékanou regions (59.71 ± 05.90 μg.g^{-1}) are higher than those measured in cocoa from other regions. Qin et al. (2017) have reported that 2-pentanol contributes to the aromatic quality of fine cocoa. The highest concentration of 2-heptanol was recorded in the cocoa from San-Pédro region. This result could probably be due to the sugars metabolism by endemic yeast species in this region (Koné et al. 2016). Valle-Epquín et al. (2020) have previously concluded that high concentration in aromatic alcoholic compounds promote the floral and sweet notes (Table 4).

Aldehydes

Table 3 shows that nonanal and benzaldehyde were the main volatile aldehydes detected in the fermented and dried cocoa beans whatever the fermentation location. Benzaldehyde differentiate cocoa beans processed in San-Pédro and Djékanou regions with highest concentration about 45 μg.g^{-1}. Nonanal was found in low concentrations ranging from 0.50 to 4.33 μg.g^{-1}. The aldehydes can result from lipid oxidation or self-oxidation and the degradation of fatty acids during the cocoa fermentation. However, Hamdouche et al. (2019) concluded that production of aldehydes is a consequence of lactic fermentation from amino acids. Whatever the pathways of the formation of aldehydes, benzaldehyde was the most abundant in the present study as previously observed by Assi-Clair et al. (2019). The altitudes of both San Pédro and Djékanou regions are comprised between those of other regions. High contents of aldehyds of cocoa from San Pédro and Djékanou regions could be explained by the geographical location and the altitude like the formation of high contents of theobromine and caffeine contents in cocoas from the Amazonian region (Júnior et al. 2020). Benzaldehyde promotes the aromatic quality of cocoa beans due to produce its pleasurable notes. So, carrying out cocoa fermentation in both San Pédro and Djekanou regions could promote the formation of aldehydes and hence the improvement cocoa products aromatic quality due to their citrus, green, fatty aroma, rose fresh, fruity, almond, cherry notes (Table 4).

Ketones

The ketone compounds highlighted in the fermented and dried cocoa grain include 2-heptanone, 2-acetoxy-3-butanone, 2, 3-butanedione, 2-nonanone and acetoin (Table 3). These results were similar to previous observations (Assi-Clair et al. 2019). Cocoa grains fermented in Daloa region recorded the highest contents of each detected ketone with the concentration is ranged from 10.63 ± 0.63 to 88.70 ± 28.50 μg.g^{-1}. Acetoin is the major ketone with the higest content as previously obtained by Valle-Epquín et al. (2020) could be produced from pyruvate and butanediol during alcoholic fermentation due to the activities of the yeasts (Bastos et al. 2018). Other studiy reported 2-heptanone as the most abundant ketone (Ho et al. 2014). The ketones concentration of cocoa grains from Daloa region was highest. The ANOVA test confirmed that the fermentation location significantly influences ($p < 0.05$) the generation of the ketone in cocoa. This influence of the fermentation location on the content and the type of ketone could be due to the yeast aromatic compounds production abilities (Koné et al. 2016). However, Assi-Clair et al. (2019) have concluded that the generation of as acetophenone, 2-heptanone, 2-pentanone was not due to the yeast metabolim. Indeed, Kadow et al. (2013) conluded that both 2-heptanone and 2-pentanone were constituent aromatic compounds of the cocoa. High contents of ketone are reported to be propitious to achieve good aromatic quality of fermented and dried cocoa. Whereas 2,3-butanedione, 2-acetoxy-3-butanone and acetoin are reported to confer buttery and creamy notes to the aromatic quality of cocoa, acetophenone, 2-nonanone and 2-heptanone produce fruity, flowery and sweet flavors (Bastos et al. 2018).

Acids

Five volatile acid were highlighted in the cocoas tested in the present study (Table 3). Both acetic and 2-methylpropanoic acids are reported to be the most abundant acids. Acetic acid is the most abundant compound with concentration comprised between 808.80 ± 219.26 and 2300.00 ± 490.56 μg.g^{-1} in cocoas from both Daloa and San Pedro regions. The formation of acetic acid was ascribed to the growth and metabolism of acetic acid bacteria (Koné et al. 2016). Otherwise, we found less acid than Rodriguez-Campos et al.

Table 4 Linear retention indices and odor description of main volatile aroma compounds identified in the cocoa seeds fermented in five fermenting regions of Côte d'Ivoire

Chemical group	Volatile compound precursors	Retention time (min)	LRI calculated	LRI**	Odor description*
Alcohols	Ethanol	6.15	904	929	Alcoholic
	2-Methyl-3-Buten-2-ol	9.98	1049	ND	Herbal
	2-pentanol	14.31	1260	1255	Green
	2-Heptanol	26.76	1329	1347	Fruity, citrus, herbal
	2,3-Butanediol	42.38	1566	1583	Sweet, flowery
	2-Phenylethyl alcohol	58.70	1889	1925	Flowery, honey
Aldehydes	Nonanal	30.70	1378	1407	Rose fresh, fruity
	Benzaldehyde	38.18	1497	1495	Sweet, almond, cherry
Ketones	2,3-Butanedione	7.25	995	963	Buttery
	2-Heptanone	17.33	1174	1170	Fruity, flowery
	Acetoin	23.60	1274	1287	Buttery, cream
	2-Acetoxy-3-butanone	30.04	1370	ND	Sweet, creamy, buttery
	2-Nonanone	30.60	1381	1388	Flowery, fatty
	Acetophenone	45.70	1622	1645	Flowery, sweet
Acids	Acetic acid	33.63	1435	1450	Sour, vinegar
	Propanoic acid	39.69	1533	1523	Purgent, rancid
	Propanoic acid, 2-methyl	41.57	1563	1568	Rancid
	Butanoic acid	45.03	1614	1619	Rancid cheese
	Butanoic acid, 3-methyl-	47.44	1664	1676	Sweaty
Esters	Isobutyl acetate	8.64	1011	1015	Fruity, banana
	Butyl acetate	11.28	1067	1075	Fruity, floral, banana
	2-Pentyl acetate	11.38	1069	1058	Fruity
	Isoamyl acetate	13.92	1117	1117	Fruity, banana
	2-Heptyl acetate	22.74	1254	ND	Woody, citrus, apricot
	2,3-Butanediol, diacetate A	36.61	1489	1486	ND
	2,3-Butanediol, diacetate B	39.01	1527	1523	ND
	Benzeneacetic acid, methyl ester	52.06	1738	ND	Honey, sweet
	Benzeneacetic acid, ethyl ester	53.60	1768	1773	Honey, flowery
	2-phenylethyl acetate	55.14	1795	1810	Honey, flowery
Pyrazines	2,3,5-Trimethylpyrazine	31.49	1395	1395	Cocoa, roasted nuts, peanuts
	2,3,5,6-Tétraméthylpyrazine	35.95	1465	1448	Cocoa roasted, chocolate
Terpène	Linalol	40.77	1554	1537	Flowery
Furanne	Furfural	35.82	1458	ND	Almond, mild, oily
Sulfur compounds	Dimethyl sulfide	3.35	685	716	Vegetable, green

ND not detected

*Aroma description from Rodriguez-Campos et al. (2012), Crafack et al. (2014), Castro-Alayo et al. (2019), Bastos et al. (2019); **obtained from literature

(2012) who detected eleven acids. Our results are similar in terms of the concentration to those previously obtained by Assi-Clair et al. (2019). The fermentation location influences the generation of aromatic acids. However, butanoic acid was the least abundant with the same level in the cocoa whatever the fermentation loctation. According to several studies, the production of butanoic acid, 3-methyl in the mucilaginous pulp was ascribed both to the acetic acid bacteria (Schwan and Wheals 2004) and Bacillus spp. (Rodriguez-Campos et al. 2012). The presence of acetic acid has been reported to confer sour notes to the aromatic quality of cocoa seeds (Afoakwa et al. 2008) whereas butanoic acide, 3-methyl was responsible for off-flavor notes according to Serra-Bonvehi (2005). Fortunately, more of the acetic acid concentration was removed by evaporation during the roasting process. Propanoic, butanoic and 2-methyl propanoic acids were described to exhibit off-flavor notes such as pungent and rancid cheese aromas due to the action of aerobic putrefactive bacteria from amino acids (Ziegleder 1991).

Esters

Table 3 showed that the esters had a greater presence in the volatile fingerprint of the tested cocoa beans. These results are quite similar to those previously obtained by Valle-Epquín et al. (2020) in Criollo cocoa bean from Peru. We found less esters than Rodriguez-Campos et al. (2012) who have highlighted twenty compounds. Isoamyl acetate, 2-phenylethyl acetate are most abundant ester differentiating the cocoa from Daloa region with 124.15 ± 88.64 and 95.70 ± 53.61 µg.g^{-1} respectively while 2, 3-butanediol diacetate A differentiate cocoa fermented in Soubré region with 160.52 ± 109.81 µg.g^{-1}. The generation of esters are ascribed to the metabolism of yeasts (Marseglia et al. 2020; Assi-Clair et al. 2019) from alcohols via esterification pathways (Rottiers et al. 2019). These reactions enzyme-catalyzed condensation between acetyl/acyl-CoA and alcohols (Rottiers et al. 2019) lead to the formation of ethyl acetate, isoamyl acetate, isobutyl acetate. However, some esters are constituent aroma compounds of the fresh cocoa bean mucilaginous. Esters were generally the second most important family of cocoa aromatic compound precursors after pyrazines. Indeed, they are responsible for fruity and floral flavors in roasted cocoa beans (Valle-Epquín et al. 2020; Rottiers et al. 2019). The geographic fermentation location significantly influences the generation of esters in cocoa.

Pyrazines

Table 3 indicates that two pyrazines namely 2,3,5-trimethylpyrazine and 2,3,5,6-tetramethylpyrazine were found in the present study. Our results are similar to those obtained previously by Assi-Clair et al. (2019) in Ivorian cocoa. The content of 2,3,5,6-tetramethylpyrazine varied from 40.30 ± 30.72 to 492.70 ± 133.93 µg.g^{-1} against from 7.55 ± 03.30 to 21.51 ± 5.78 µg.g^{-1} for 2,3,5-trimethylpyrazine. This result is in agreement with those obtained by Rottiers et al. (2019). Both detected pyrazines differentiate cocoa from Daloa region with the highest concentration. Pyrazines originate by microbial metabolism during fermentation and their concentration increased during roasting especially by Strecker degradation that accompanies the Maillard reaction (Marseglia et al. 2020; Valle-Epquín et al. 2020). Table 4 reported that pyrazines are the most important of the volatile components in cocoa due to their greatest chocolate notes (Valle-Epquín et al. 2020). Hence, our cocoas present a great potential for production of chocolate with the best sensory properties after raosting (Valle-Epquín et al. 2020). According to Koné et al. (2016), the presence of pyrazines is also a great indicator for both the presence of beneficial yeast strains and a good fermentation degree. Cocoa fermentation location significantly influences pyrazines generation in cocoa seeds as previous observed by Hamdouche et al. (2019) via the metabolism of Bacillus subtilis.

Others volatile compound precursors

Table 3 show that other volatile compounds including terpenes (linalool), furanes (furfural) and sulfur compound family (dimethyl sulfide). These results are in disagreement with those previously detected 2-methoxy phenol, 2-acetyl-1H-pyrrole and phenol (Rodriguez-Campos et al. 2012). Linalool, fufural and dimethyl sulfide were found at low concentrations below 4 µg.g^{-1} in all cocoa bean samples whatever the tested fermentation locations. However, cocoa beans from Djékanou region exhibited high concentration in linalool. This result is in accordance with those of various studies (Valle-Epquín et al. 2020; Ziegleder, 1991) who found it in roasted cocoa. Furfural and dimethyl sulfide were reported to confer desirable flavors to cocoa end-chocolate (Table 4) while linalool was reported to be a good odor-active compound greatly influencing the sensory quality of the chocolate (Qin et al. 2017). Castro-Alayo et al. (2019) have concluded that the formation of linalool is ascribed to the leucine catabolism under yeast activities. The cocoa fermentation location via the diversity of the yeasts strains significantly influences the generation of other volatile compounds.

Impact of fermentation location on the fingerprint of volatile compounds precursors of cocoa

To clearly visualize the relationship between cocoa fermentation region and the formation of flavor compounds in cocoa grains, PCA was performed (Fig. 1). The biplot shows that the first two principal components (PC) explained 76.31% of the total variability. F1, accounting for 50.29% of total variance, discriminated the cocoa beans processed at the San Pédro region from those treated at the regions of Akoupé, Djékanou, Daloa, Soubré. The cocoa bean samples in former group (negative axis) treated at the San-Pédro region were characterized by the high content in less numerous (8) aromatic compound precursors (2-heptanol, 2-nonanone, 2-heptanone, nonanal, benzaldehyde, 2-heptanol acetate, methyl benzeneacetate and butanoic acid, 3-methyl) than those processed at other fermentation location in the second former group (positive axis). F2, explaining 26.03% of the variability, highlighted the differences between the detected aromatic compound precursors. The former group (negative axis) consists in two fermentation locations including Djékanou and Soubré (Fig. 1). The cocoa samples treated there were characterized by 12 aromatic compound precursors such as 2-pentanol, acetate, 2,3-butanediol, 3-buten-2-ol, 2-methyl, benzeneacetic,

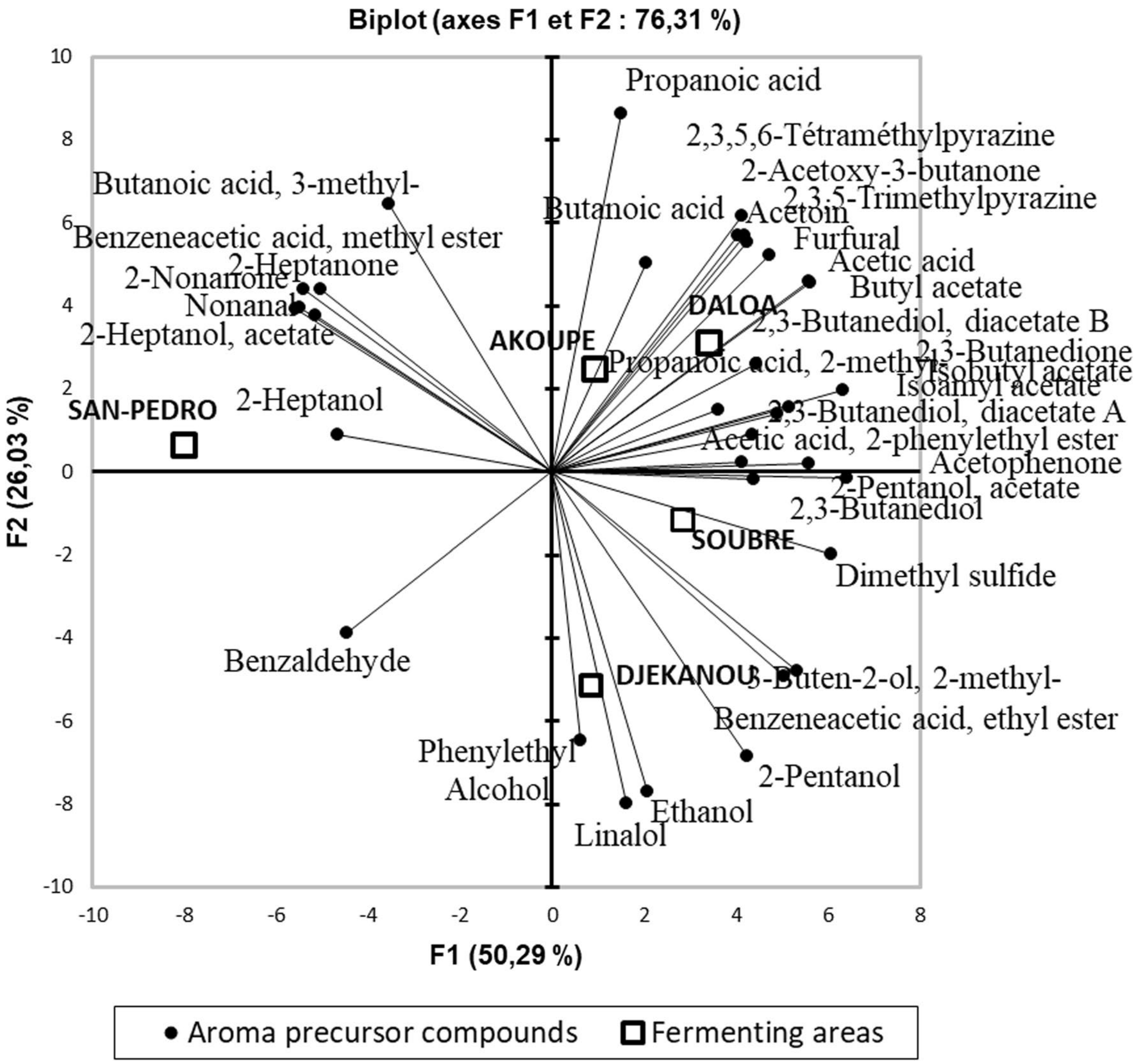

Fig. 1 Principal component analysis (PCA) bi-plot showing aroma precursor compounds of raw cocoa samples as affected by different Ivorian fermenting sites

ethyl ester, 2-pentanol, ethanol, linalool, phenyl ethyl alcohol, benzenaldehyd and dimethyl sulfide. The second former group (positive axis) combined a large number (20) of aromatic compound precursors highlighted in the cocoa seeds processed at the regions of Daloa, Akoupé and San Pédro. The cocoa beans processed at the Daloa region recorded the highest contents of desirable aromatic compound precursors i.e. esters, ketones, and aldehydes. So, the differences between the aromatic compound precursor profile detected in cocoa beans in function of the fermentation location could be ascribed to the endemic microbiota (yeast in particular) as previously conluded (Maura et al. (2016). Among five main groups of microorganisms participating in cocoa fermentation, the contribution of bacteria and yeasts to the generation of aromatic compound precursors were clearly highlighted (Rodriguez-Campos et al. 2012). However, recent research has reported the most important roles of yeasts in the generation of aromatic compounds of chocolate flavor. Probably S. cerevisiae and P. kudriazevii produce a wide variety of desirable aromatic compound precursors (Junior et al. 2021) are well-adapted and relevant yeast species at the Daloa region as previously observed in Agneby Tiassa an other big cocoa producing region in Côte d'Ivoire (Koffi et al. 2017).

Sensory attributes of finished chocolate

Fifteen descriptive attributes namely intensity of odor, sweet, sour, bitter, astringent, cocoa aroma, fresh fruit, dried fruit, floral, vegetable, spicy, alcoholic aroma, animal, cocoa odor and global quality were evaluated. Average of the score attributed for each sensory property to the

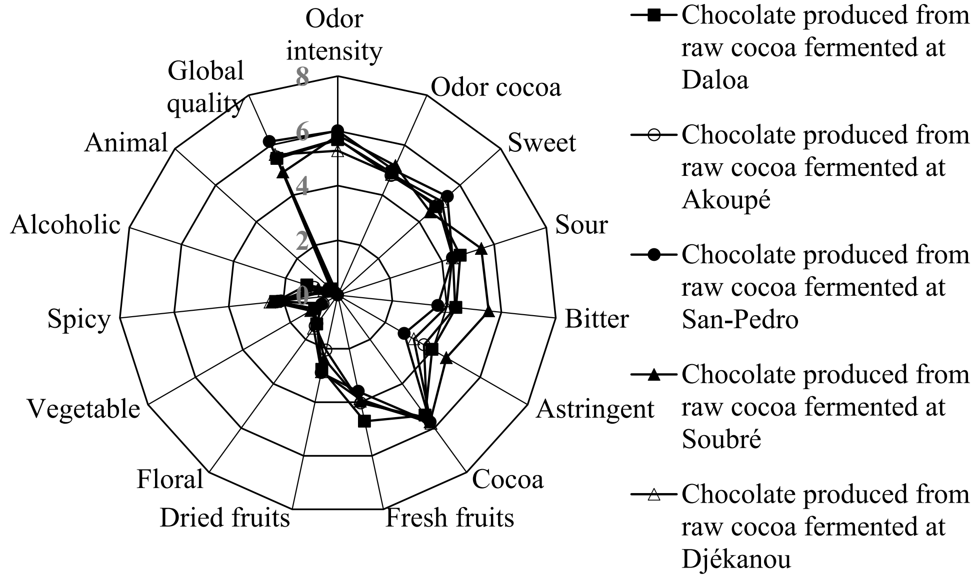

Fig. 2 Sensory traits of chocolate produced from cocoa grains processed according to the major cocoa producing regions as fermenting sites at Côte d'Ivoire

finished chocolates from each fermented cocoa sample was then calculated. Figure 2 shows that all finished chocolates present the same score for spicy and cocoa odor notes. These sensory descriptors could probably be the result of the combination of flavors due to the constituent aromatic compound precursors. The spicy flavor could be promoted by the genotype and the growing conditions of the cocoa tree (Bastos et al. 2019). Chocolates produced from the cocoa from Daloa region recorded the highest score (5.4) for fresh fruit notes. Highest score of sour (5.8), astringent (5.2) and bitter (5.8) notes affect chocolate made from the cocoa sourced from Soubré region. The highest scores for both floral and fresh fruit traits attrubted to chocolates issued from cocoa fermented in Daloa region could be due to the highest concentrations of alcohols, ketones and esters (Assi-Clair et al. 2019). Junior et al. (2021) have highlighted the ability of yeast for to production of esters, alcohols and aldehydes. These observation suggest the presence of high potential aromatic compound precursors producing yeast (Koné et al. 2016) among the endemic microbiota of the Daloa region. Finished chocolates from cocoa from San Pédro region recorded the highest score (6.2) for the global sensory quality. However, the key aromatic compound precursor content of the cocoa from San Pedro were low. This allows us to confirm that the roasting operation of cocoa beans improve deeply the sensory perceptions of cholate as Valle-Epquín et al. (2020) previously concluded. The highest scores for the undesirable flavors (sour, bitter and astringent notes) differentiating chocolates made from cocoa fermented in both Soubré and Daloa regions could be due to the high contents of acid (Bastos et al. 2019). Consequently, no intimate relationship exists between the concentration of aromatic compound precursors in cocoa seeds most generated during the fermentation and the sensory attributes of chocolate originate by the roasting step (Janek et al. 2016). Assi-Clair et al. (2019) have shown that the organoleptic attributes of chocolate flavor are not due only to the volatile profile but to both volatile and non volatile fractions. That's why Kongor et al. (2016) concluded that the concentrations and types of volatile compound precursors originated by microbiological processes are not a good indicator for cocoa aromatic quality. However, Valle-Epquín et al. (2020) confirmed that only roasting determines both the true fingerprint of aromatic compounds of cocoa grains and the sensory perceptions of chocolate produced thereof.

Conclusion

The fermentation location did not influence the physical quality of cocoa beans. However, the geographic origin and altitude of cocoa region significantly influenced the generation of aromatic compound precursors in cocoa beans probably via the diversity of endemic microorganisms. Our study showed that a good fermentation process will ensure good aromatic quality of cocoa beans in term of genertation of aromatic compounds precrusors. Among all studied regions, San-Pédro region appears to be the most favorable fermentation location for the best fingerprint of volatile compounds precursors in high contents. However, it is certain that the best sensory properties of finished chocolates are orginate by both fermentation and roasting processes. This study was limited by the lack of both data on the sensory perceptions of chocolate and the microbial ecology according to the fermentation location. These consequent and interesting yeast data will be published specially in the separate publication in the near future. An analysis of aromatic compounds of the finished chocolates will be made to know its variation during the industrial process of making the chocolates. This should be confered an effect "Terroir" to the cocoa sourced from Côte d'Ivoire.

Acknowledgements This research was funded by "Service de Coopération et d'Action culturelle" at the French Embassy in Abidjan, Côte d'Ivoire through a Ph'D grant to DAK. The authors thank Mr and Mrs Anon, the cocoa farming family at Akoupé, a region located in the southeast of Côte d'Ivoire for providing the cocoa pods to perform the cocoa fermentation tests in the selected cocoa fermenting regions in Côte d'Ivoire.

Funding The authors have not disclosed any funding.

Declarations

Conflict of interest The authors have not disclosed any competing interests.

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