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Total Fat, Fatty Acid Composition, Tocopherol and Tocotrienol Profiles in Fermented Beans of Ten Controlled Pollinated Cocoa (*Theobroma cacao* L.) Hybrids from Cameroon

Martin Paul Arnaud Mbida[1,2], Simon Perrez Akoa[2,3], Ronelle Fabiola Mbia[2,4], Manga Ndjaga Jude[2], Martine Louise Ondobo[2], Onomo Pierre Effa[1,2,5*]

[1]Department of Biochemistry, Faculty of Science, University of Yaounde I, Yaounde, Cameroon
[2]Laboratory of Plant Physiology and Biochemistry, Department of Biological Science, Higher Teacher's Training College, University of Yaounde I, Yaounde, Cameroon
[3]Department of Food Science and Technology, Faculty of Agriculture and Veterinary Medecine, University of Buea, Buea, Cameroon
[4]National Technology Development Committee, Ministry of Scientific Research and Innovation, Administrative Center, Yaounde, Cameroon
[5]Department of Agriculture and Agropastoral, Higher Technical Teacher's Training College, University of Ebolowa, Ebolowa, Cameroon
Email: *peffafr@yahoo.fr

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Abstract

This study aimed to discriminate ten Cameroonian cocoa hybrids according to their total fat, fatty acid composition, tocopherol and tocotrienol profiles. Six cocoa clones from the gene banks of the Cameroon Cocoa Development Corporation were used to create hybrids. The determination of fatty acid composition was carried out by using a gas chromatography (GC) apparatus coupled by a flame ion detector (FID). Tocopherol and tocotrienol analysis was performed by upper high-performance liquid chromatography (UHPLC). Information on the impact of the genotype on the cocoa fat composition was provided. The major fatty acids (FA) in fermented samples are stearic (34.57%), palmitic (26.13%), oleic (34.13%) and linoleic (3.16%) acids. (35.05% to 35.6%). SCA12 × ICS40, SCA12 × SNK13, SNK13 × T79/501 have the least hard cocoa butters. Tocopherols analysis showed a predominance of γ-tocopherols (94.64 ± 1.51 to 292.16 ± 3.17 $\mu g \cdot g^{-1}$), whereas only a small amount of β and δ-tocopherol (from 0.46 to 2.78 $\mu g \cdot g^{-1}$ and 0.12 to 5.82 respectively) was observed. No γ-tocotrienol was found in fermented samples. A differentiation in terms of total fat and tocopherol content was observed amongst hybrids with the same mother-clone, suggesting an impact of pollen on these compounds.

Keywords

Cocoa Hybrids, Lipid Composition, UHPLC, GC-FID

1. Introduction

Cocoa (*Theobroma cacao* L.) is one of the world's most valuable crops and attractive food with great economic significance [1]. The main cocoa bean producing countries are African countries (mainly Ivory Coast, Ghana, Cameroon and Nigeria) supplying around 76% of the global production in 2023 [2]. Cocoa is a nutraceutical used in cosmetic and agri-food industries, and particularly in chocolate production. The essential ingredient that forms the only continuous fat phase in chocolate is known as cocoa butter (CB). This natural fat is highly appreciated and is expensive compared with all other vegetable fats and oils because of its specific characteristics such as pale yellow colour, neutral taste, and sharp melting profile, body-temperature alike [3]. CB is also one of the most precious and useful vegetable fats obtained from cocoa beans and widely used in both cosmetics and food preparation [4]. There is no other naturally occurring fat with the same physical properties as CB [5]. Fats represent approximately 30% - 50% of cocoa beans chemical constituents [6]. The high-energy values of the cocoa are almost entirely derived from the fat content. One gram of lipid provides around 9 $kcal \cdot g^{-1}$, as compared to protein and carbohydrate each yielding around 4.5 $kcal \cdot g^{-1}$. The fat content of cocoa consists mostly of palmitic acid, oleic acid, and stearic acid forming a selection of triacylglycerols (TAG). Fats are important nutrients for human wellbeing. In human diets, lipids act as a heating medium for food processing and affect the texture, mouth feel and flavour of foods [7]. It has been showed that saturated fatty acids (palmitic or stearic acids) are important constituents for reserve triglycerides, phosphoglycerolipids and sphingolipids. Monounsaturated fatty acids (oleic acid) are known as cardiovascular protector elements. Indeed, they are recognized to drop down bad cholesterol (LDL) and enhance the formation of good cholesterol (HDL). Polyunsaturated fatty acids (linolenic ω-6 and linoleic ω-3 acids) are rarely synthetized and are known as indispensable because they must be obtained by foods. These involved in many physiological functions as energy supply, prostaglandin, and thromboxane synthesis, etc... [8]. It was previously suggested that soft cocoa butters are characterized by higher 1-palmitoyl-2,3-dioleoyl-glycerol, 1-stearoyl-2,3-dioleoyl-glycerol content [5] [9] whereas hard cocoa butters are characterized by increased saturated fatty acid (SFA) content. Free fatty acids (FFA) are released from TAG [10] through the effect of a lipase or an oxidation. However, the risks of oxidation are negligible in cocoa butter due to its high content of polyphenols, natural antioxidants, and vitamin E in cocoa beans. Vitamin E refers to a group of eight different compounds including alpha (α), beta (β), gamma (γ), and delta (δ) tocopherols and tocotrienols [11]. Although they are

not the major components in cocoa butter, their presence is essential for the stability of unsaturated fatty acids, preventing oxidation reactions [12]. In fact, Vitamin E is an important antioxidant, which, due to a chroman group, halts lipid peroxidation by donating its phenolic hydrogen to peroxyl radicals forming tocopheroxyl radicals that are unreactive and unable to continue the oxidative chain reaction [13] [14]. The origin can have an impact in cocoa beans composition for several reasons [7]. Chocolate manufacturers spend significant effort on sourcing raw material with specific characteristics from a particular country. Some authors [15] have showed that climate conditions at the geographical areas of cultivation have significantly influence on bean chemical composition and quality. The quality of beans is also ensured by the quality of fermentation, essential step during the cocoa beans processing [16] [17] [18]. CB can be derived from either fermented or unfermented cocoa beans. [19] demonstrated that fermentation time caused minimal changes in fatty acid (FA) levels of the Ghanian cocoa beans. In the same order, [20] showed that fermentation slightly affect the profile of FA from various countries (Ivory Coast, Tanzania, Indonesia, Ecuador, Malaysia, and Brazil). In addition, [21] founded that with increasing fat content in dark chocolate, the perceived intensities of bitter taste, cocoa flavor, and drying mouthfeel all decreased, while sweetness increased. In Cameroon, the 5th largest cacao producer, cocoa varieties from the three major groups are cultivated [22]: Criollo (fine cocoa flavour), Forastero (bulk cocoa except a few such as *Scavina*) and Trinitario (cocoa with some interesting aromas), with the predominance of the last two varieties. Cameroon is otherwise the largest African producer of Trinitario cocoa [23]. However, some authors highlighted many incompatibility cases between Trinitario clones [24]. Despite this, [25] shown that breeding between Trinitario and Forastero offer hybrids with interesting agronomic and physicochemical parameters. Cameroon's cocoa hybrids are priced for their characteristic reddish colored cocoa powder sought after by the baking, biscuit and ice cream industries [26] but they are poorly investigated in terms of the fat contents and vitamin E profile. The aim of this work was to assess total fat, tocopherol and tocotrienol profiles for their potential usefulness as markers in separating traditional cocoa groups in Cameroon.

2. Material and Methods

2.1. Reagents and Standards

All solvents used were of analytical or HPLC grade and supplied by Merck (Darmstadt, Germany). Water was purifed in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Standard compounds such as α, γ, δ-tocopherol (≥99%) and γ-tocotrienol (≥97%), were purchased from Sigma-Aldrich (St. Louis, MO, USA). The FA methyl ester (FAME) standards were obtained from Supelco (Bellefonte, USA).

2.2. Cocoa Plant Material

Ten cocoa hybrids were obtained by hand pollination from six parental clones:

two local Trinitario (SNK13 and SNK413), one Trinitario introduced from Trinidad (ICS40), and three Forestero (SCA12, UPA134 and T79/501) from the gene banks of the Cameroon Cocoa Development Corporation (SODECAO) at the Mengang Station (situated at 82 km from Yaounde; 3˚53' NL, 12˚03' EL in the centre region of Cameroon). Mengang station falls under a bimodal forestry zone recording an annual rainfall of 1500 - 2000 mm and an average temperature of 25˚C. These clones were selected for their tolerance to *Phytophthora megakarya* and their productivity [27] [28]. Crossings were conducted over two cocoa crop years (from February 2 to November 21, 2017 and from February 9 to November 29, 2018) as describe by [25].

2.3. Post-Harvest Treatment of Cocoa Beans

One thousand ripe cocoa pods from different hybrids were harvested from the experimental plots of the SODECAO at Mengang Station. After two days of pod storage, the ripe pods were split and beans obtained were fermented using the box method. The fermentation was done by putting the extracted cocoa beans in the boxes. The beans were covered with banana leaves and fermented for six days with opening and overturning every two days. The fermented cocoa beans were then lyophilized.

2.4. Determination of Total Fat Content

Total fat content was evaluated by continuous extraction by the Soxhlet method using hexane as solvent [29]. The extract was filtered and the hexane removed by evaporation at 50˚C under vacuum. Then, the obtained fats were poured into dark glass bottles, hermetically sealed and stored at −18˚C to avoid glycerides and minor compounds oxidation and/or degradation, until analyses. The result was reported in grams of fat per 100 g of dry weight ($g.100g^{-1}$ DW).

2.5. FAME Synthesis for Fatty Acids Analysis

Fatty acid methyl esters (FAME) were prepared using the standard method according to ISO E. 5509 (2000) with modifications. 0.7 mL 10 N KOH, 5.3 mL methanol and 100 mg initial lipid extract were stirred continuously at 50˚C for one hour. 500 µL of toluene was added together with BF_3/CH_3OH, to allow good dissolvement of lipid extracts. The samples were cooled down at room temperature and 0.58 mL 24 N H_2SO_4 was added. All samples were stirred continuously at 50˚C for one additional hour. FAME were extracted with 2 mL cyclohexane, mixed and centrifuged at 9000 g for 5 minutes at 10˚C. Molecular sieves were added for 10 minutes in the final FAME solution to further neutralize the mixture. Then solution was diluted and nitrogen was blown on every sample to avoid oxidation and then the samples were directly analysed by gas chromatography.

2.6. GC-FID Method

FAME were determined according to the gas chromatography method described

by [30] by capillary gas chromatography on Agilent Technologies 6890 (USA) GC device equipped with split/splitless injector, flame ionization detector (FID) and capillary column Supelco SP-2560 (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, Supelco, Bellefonte, USA). Injector and detector temperatures were 250˚C and 260˚C, respectively. Helium was used as the carrier gas at a flow rate of 5 mL·min^{-1}. The injected volume was 1 µL and the injector split ratio was set at 20:1. The column temperature was programmed from the initial 50˚C (held 5 min) to 240˚C (held 20 min), with a temperature rate of 4˚C·min^{-1}. The chromatographic peaks in the samples were identified by comparing the relative retention times of FAME peaks with peaks in Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA). Indeed, the content of methyl esters of fatty acids (MEMK) is determined, and the relative content of individual fatty acids (FA) is determined (calculated) accordingly.

2.7. Tocopherols and Tocotrienols Analysis by UHPLC

Pure tocopherol (used as standards of tocopherol and tocotrienol) was diluted in hexane (HPLC grade), at a concentration of a 10 µg·mL^{-1}. The analysis was performed using an UHPLC binary system (Dionex Ultimate 3000 RSLC, Waltham, MA, USA) equipped with a binary pump (HPG 3200RS), auto-sampler (WPS 3000), column compartment (TCC 3000) and the Chromeleon software for data acquisition and processing. A Nova-Pack C18 analytical column (150 × 4.6 mm i.d.) with a particle size of 2.2 µm (INTERCHIM, Uptisphere strategy SI) was used. Briefly, 80 mg of cocoa butter were dissolved in 1 mL of hexane/isopropanol (3:2 v/v), vortexed, filtered through 0.45 µm pores filters and then injected into the HPLC system. The mobile phase consists of a mixture of acetonitrile and methanol (1:1, v/v) at a constant flow rate of 1 mL·min^{-1} in isocratic elution. The injection volume was 20 µL. The FLD (Dionex 3400RS, Waltman, MA, USA) spectrofluorometer was used as detector, set at excitation wavelength of 295 nm and emission at 325 nm, using external standard calibration curves of α, γ, δ-tocopherol and γ-tocotrienol. Total tocopherol content was calculated as the sum of the identified and quantified tocopherols and expressed as µg·g^{-1} of lipid extract.

2.8. Statistical Analysis

The values are means of three replications. Results were analyzed with variance (ANOVA) followed by Tukey HSD at $P < 5\%$ to compare means with the assistance of SPSS 20.0 for windows. Principal component analysis (PCA) was performed to establish associations among tocopherols and fatty acids by using SPAD 5.5 software package. Excel software was used to draw some figures.

3. Results and Discussion

3.1. Total Fat (TF) Content

The average TF content of the different fermented cocoa hybrids varied between

44.54% ± 1.37% and 59.32% ± 1.83% (Table 1). This highest value was recorded by T79/501 × SNK13 (Forastero female-clone). All the hybrids, except SCA12 × ICS40 and SCA12 × UPA134, were distinguished through an average fat content above 50.00%. [31] reports that in Criollo cocoa, the fat content tends to be lower than Forastero, estimated to be approximately 53%. Nonetheless, SCA12 × ICS40 and SCA12 × UPA134 (both Forastero mother-clone) recorded an average fat content of 44%. In addition, results obtained for different hybrids were close to the ranges reported by several authors for fine cocoa samples [4] [32] [15] [33]. The variations in the fat content of cocoa beans could be related to several factors such as environmental conditions, growing area [15] [31] and genotype. For this purpose, in this study, significant differences ($P < 0.05$) in terms of TF content were observed amongst hybrids with the same mother-clone, suggesting an impact of pollen origin. [25] assumed that, allopollen grains induce an increase in beans weight and the female-clone organizes itself to create an adequate environment for the expansion of seeds. We can suggest that this expansion is caused in part by increasing the fat content. On the other hand, [34] highlighted that the cacaos with highest market value, are those with fat contain above 56%. In this sense, only SNK413 × SCA12 (Trinitario female-clone) and T79/501 × SNK13 can be considered as having high commercial value.

3.2. Fatty Acid Composition

The fatty acid (FA) composition in fermented samples is illustrated in Figure 1 and Table 2. The major FA according to their average content are stearic (34.57%), palmitic (26.13%), oleic (34.13%) and linoleic (3.16%) acids. The saturated fatty acids (SFA) content ranged between 59.25% ± 0.78% (for SCA12 × ICS40) and 63.7% ± 0.21% (for SCA12 × UPA134) while polyunsaturated fatty

Table 1. Fat contents in fermented beans of ten Cameroonian cocoa hybrids.

Hybrids	Total fat content in % (g.100 g^{-1})
ICS40 × SCA12	54.05 ± 0.37^{cd}
SNK13 × ICS40	50.82 ± 0.52^{c}
SNK13 × T79/501	53.68 ± 1.41^{cd}
SNK413 × SCA12	56.12 ± 0.52^{d}
SNK413 × T79/501	53.80 ± 1.38^{cd}
SCA12 × ICS40	44.98 ± 2.05^{a}
SCA12 × SNK13	52.11 ± 5.04^{cd}
SCA12 × UPA134	44.54 ± 1.37^{a}
T79/501 × SNK13	59.32 ± 1.83^{de}
T79/501 × SNK413	46.68 ± 0.93^{ab}

Values with the same letter are not significantly different ($P < 0.05$, Tukey HSD)

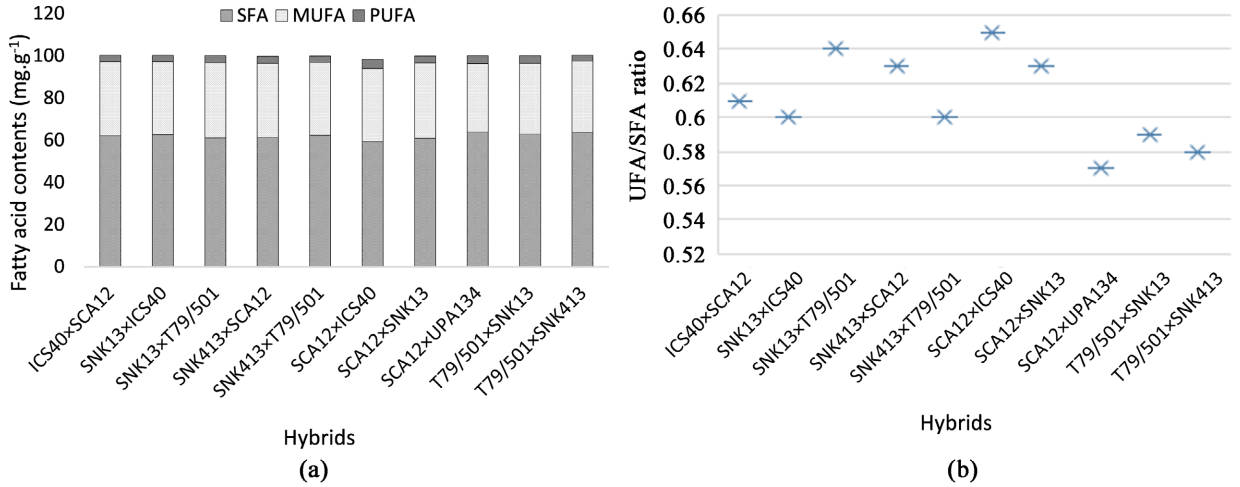

Figure 1. Global Fatty Acid composition in fermented beans of ten cocoa hybrids from Cameroon. (a) Fatty (Saturated, Monounsaturated and Polyunsaturated) acid contents; (b) Unsaturated fatty acids/Saturated fatty acids ratio.

Table 2. Relative contents (%m/m) of fatty acids identified in fermented beans of 10 cocoa hybrids.

Hybrids / FA	ICS40 × SCA12	SNK13 × ICS40	SNK13 × T79/501	SNK413 × SCA12	SNK413 × T79/501	SCA12 × ICS40	SCA12 × SNK13	SCA12 × UPA134	T79/501 × SNK413	T79/501 × SNK13
Myristic acid C14:0	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}	0.15 ± 0.06^{a}	0.75 ± 0.05^{b}	0.10 ± 0.00^{a}	0.10 ± 0.01^{a}	0.10 ± 0.00^{a}	0.10 ± 0.00^{a}
Palmitic acid C16:0	22.7 ± 1.04^{a}	26.50 ± 1.96^{b}	27.2 ± 1.35^{b}	28.55 ± 1.44^{c}	24.05 ± 0.06^{ab}	28.75 ± 1.56^{c}	28.30 ± 0.69^{c}	24.8 ± 0.81^{ab}	24.9 ± 0.92^{ab}	25.55 ± 0.52^{ab}
Stearic acid C18:0	38.3 ± 0.29^{de}	34.80 ± 0.92^{c}	32.55 ± 0.64^{b}	31.60 ± 1.58^{b}	37.05 ± 0.52^{d}	28.45 ± 1.21^{a}	31.55 ± 0.17^{b}	37.70 ± 0.23^{d}	37.6 ± 0.29^{d}	36.15 ± 0.06^{d}
Arachidic acid C20:0	0.85 ± 0.17^{a}	0.95 ± 0.12^{a}	0.90 ± 0.14^{a}	0.90 ± 0.12^{a}	0.75 ± 0.07^{a}	0.85 ± 0.06^{a}	0.80 ± 0.11^{a}	0.90 ± 0.12^{a}	0.90 ± 0.12^{a}	0.80 ± 0.12^{a}
Behenic acid C22:0	0.10 ± 0.02^{a}	0.20 ± 0.01^{a}	0.20 ± 0.02^{a}	0.15 ± 0.06^{a}	0.25 ± 0.07^{ab}	0.15 ± 0.05^{a}	0.10 ± 0.00^{a}	0.20 ± 0.03^{a}	0.15 ± 0.03^{a}	0.15 ± 0.03^{a}
Palmitoleic acid C16:1	0.20 ± 0.02^{a}	0.20 ± 0.00^{a}	0.20 ± 0.01^{a}	0.15 ± 0.06^{a}	0.15 ± 0.05^{a}	0.25 ± 0.08^{ab}	0.15 ± 0.06^{a}	0.10 ± 0.02^{a}	0.10 ± 0.01^{a}	0.15 ± 0.07^{a}
Oleic acid C18:1	34.7 ± 0.17^{ab}	34.1 ± 0.35^{ab}	35.3 ± 0.35^{ab}	34.75 ± 0.40^{ab}	34.15 ± 0.17^{ab}	34.15 ± 0.64^{ab}	35.2 ± 0.20^{ab}	32.30 ± 0.12^{a}	33.5 ± 0.12^{a}	33.2 ± 0.11^{a}
Gadoleic acid C20:1n-9	0.05 ± 0.00^{a}	0.10 ± 0.00^{a}	0.10 ± 0.03^{a}	0.05 ± 0.01^{a}	0.10 ± 0.12^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.10 ± 0.02^{a}	0.05 ± 0.00^{a}
Erucic acid C22:1n-9	0.10 ± 0.01^{a}	0.20 ± 0.02^{ab}	0.10 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.01^{a}	0.20 ± 0.04^{ab}	0.05 ± 0.01^{a}	0.10 ± 0.02^{a}	0.10 ± 0.03^{a}	0.15 ± 0.06^{ab}
Linoleic acid C18: 2n-6	2.95 ± 0.17^{a}	2.85 ± 0.06^{a}	3.25 ± 0.17^{b}	3.16 ± 0.40^{b}	2.85 ± 0.17^{a}	4.05 ± 0.30^{c}	3.10 ± 0.17^{b}	3.45 ± 0.17^{b}	2.60 ± 0.12^{a}	3.35 ± 0.10^{b}
α-Linolenic acid C18:3n-3	0.10 ± 0.00^{a}	0.15 ± 0.06^{a}	0.25 ± 0.08^{ab}	0.25 ± 0.05^{ab}	0.25 ± 0.06^{ab}	0.30 ± 0.09^{ab}	0.25 ± 0.07^{ab}	0.25 ± 0.06^{ab}	0.20 ± 0.01^{ab}	0.25 ± 0.06^{ab}

Values with the same letter in the same line are not significantly different ($P < 0.05$, Tukey HSD).

acids (PUFA) varied from 2.8% ± 0.15% (for T79/501 × SNK413) to 4.35% ± 0.10% (for SCA12 × ICS40). SNK13 × T79/501 (hybrid with Trinitario female-clone) and SCA12 × SNK13 recorded the highest content of monounsaturated fatty acid (MUFA). [35] also noted that the chemical fatty acid composition of cocoa butter is predominantly made up of saturated fatty acids, followed by the monounsaturated ones. In general, the fatty acid composition obtained is in agreement with the results reported by [15]. These authors found average contents of palmitic (27.6%), stearic (33.8%) and oleic (34.7%) acids in Ecuadorian cocoa (known as fine cocoa) samples. The similarity between these results can suggest that our hybrids have characteristic features of fine cocoas, especially as [36] reported the similar composition of MUFA (34.06%) in dark-chocolate samples with fine Criollo cocoa from Mexico. The major MUFA in all the hybrids is oleic acid (32.3% ± 0.12 to 35.3% ± 0.20%). These one play an important role in the prevention and treatment of cardivascular diseases through different mechanisms [37]. In this sense, SNK13 × T79/501 and SCA12 × SNK13 having the healthiest FA profile. Furthermore, significant differences in terms of linoleic acid content were observed amongst hybrids with the same mother-clone ($P < 0.05$). The same observation was done for stearic acid content except between T79/501 × SNK13 and T79/501 × SNK413. Further investigations are needed to elucidate the role of pollen grain on the profile of these two particular fatty acids in hand-pollinated cocoa hybrids. On the other hand, others fatty acids (palmitoleic, erucic, gadoleic and *α*-linoleic acids) have been identified in trace amounts (<1 %) in our samples. This result is in agreement with those of [20]. According to her findings, the quantity of palmitoleic acid, linoleic acid and unusual fatty acids could be considered as markers of the fermentation status. The very low erucic acid content is an asset for the nutritional quality of cocoa butter because erucic acid is thought to be involved in harmful effects on the cardiovascular system. Although fermentation has shown to have a significant impact on the lipid profile of cocoa [20], differences in the composition of the fatty acids profile among the fermented samples can be mainly explained by the effect of the cocoa genotype which is linked to pollen genetic origin. In addition, the UFA/SFA ratio varied from 0.57 (SCA12 × UPA134) to 0.65 (SCA12 × ICS40). This ratio determines the degree of hardness [38] and the melting properties [36] of cocoa butter. The lower this ratio, the harder the butter and the lower the melting point. This is explained by the fact that TAG with saturated fatty acids forms a more compacted structure, which is more difficult to melt. According to this point of view, SCA12 × ICS40, SCA12 × SNK13, SNK13 × T79/501 and SNK413 × SCA12 have the least hard cocoa butters.

3.3. Tocopherol and Tocotrienol Contents

The concentration of total tocopherols (**Figure 2**) ranges from 96.34 to 306.75 $\mu g \cdot g^{-1}$ of lipid extract of fermented cocoa samples. The results of tocopherol and tocotrienol analysis **Table 3** showed a predominance of *γ*-tocopherols (94.64 ±

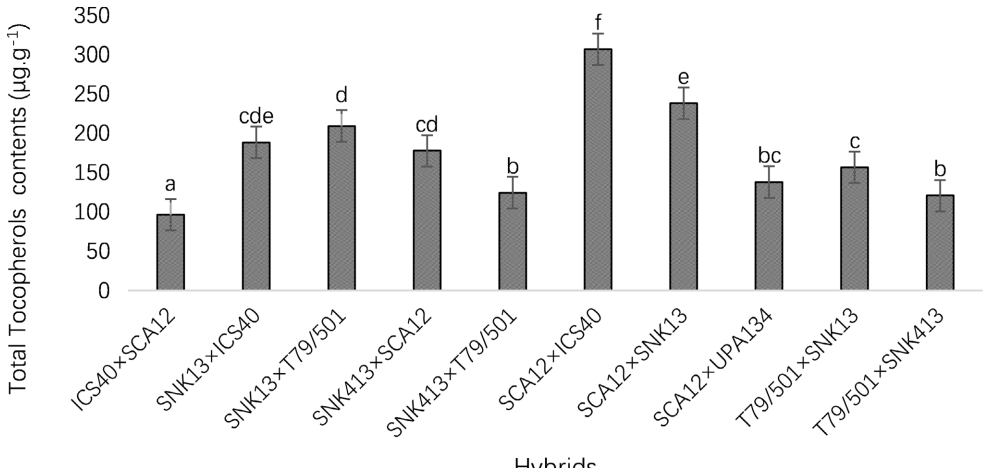

Figure 2. Total tocopherols contents in fermented beans of ten Cameroonian cocoa hybrids.

Table 3. Tocopherol and tocotrienol contents in ten selected cocoa hybrids (in µg·g^{-1} of lipid extract).

Hybrids	*γ*-tocotrienol	*α*-tocopherol	*β*-tocopherol	*γ*-tocopherol	*δ*-tocopherol
ICS40 × SCA12	nd	nd	1.70 ± 0.10^{cd}	94.64 ± 1.51^{a}	nd
SNK13 × ICS40	nd	nd	0.46 ± 0.27^{a}	187.91 ± 2.42^{d}	nd
SNK13 × T79/501	nd	1.88 ± 0.07^{b}	1.76 ± 0.86^{d}	204.28 ± 3.04^{de}	1.26 ± 0.09^{c}
SNK413 × SCA12	nd	0.32 ± 0.10^{a}	1.62 ± 0.28^{cd}	174.45 ± 2.05^{cd}	1.23± 0.28^{c}
SNK413 × T79/501	nd	nd	1,45± 0.03^{c}	122,84 ± 3.05^{b}	0.14± 0.17^{a}
SCA12 × ICS40	nd	5.99 ± 0.06^{c}	2.78 ± 0.17^{de}	292.16 ± 3.17^{f}	5.82 ± 0.87^{e}
SCA12 × SNK13	nd	2.67 ± 0.23^{bc}	0.89 ± 0.07^{b}	232.85 ± 2.74^{e}	1.82 ± 0.56^{d}
SCA12 × UPA134	nd	nd	0.84 ± 0.17^{b}	136.81 ± 2.38^{bc}	0.33 ± 0.47^{ab}
T79/501 × SNK13	nd	nd	2.97 ± 1.14^{e}	152.97 ± 2.45^{c}	0.74 ± 0.53^{b}
T79/501 × SNK413	nd	nd	1.66 ± 0.23^{cd}	118.99 ± 2.03^{b}	0.13 ± 0.23^{a}

Values with the same letter in the same column are not significantly different ($P < 0.05$, Tukey HSD) nd: not detected.

1.51 to 292.16 ± 3.17 µg·g^{-1}), whereas only a small amount of *β* and *δ*-tocopherol (from 0.46 to 2.78 µg·g^{-1} and 0.12 to 5.82 µg·g^{-1} respectively) was observed. An absence of α-tocopherols is also observed in three hybrids with Trinitario mother-clone (SNK13 × ICS40, SNK413 × T79/501 and ICS40 × SCA12) and three hybrids with Forastero mother-clone (SCA12 × UPA134, T79/501 × SNK413 and T79/501 × SNK13) unlike the others hybrids. The *β*-tocopherol content, although being minor, was comparable with that of some common edible oils [38]. The amounts of *δ*-tocopherol obtained in this study were lower than those reported by [39] in Quercus oils. On contrary, the *β*-tocopherol was found in higher amount followed by *γ*-tocopherol in another study conducted by [40]. It is well known that tocopherols are natural antioxidants and their presence in oilseeds is often correlated with a relative abundance of UFA [41].

Indeed, tocopherols play a role in protecting MUFA and PUFA from oxidation [38]. SCA12 × ICS40 showed the highest tocopherol content whereas his reciprocal hybrid ICS40 × SCA12, had the lowest content of both the γ-tocopherols and the total tocopherol content. This result proves a qualitative improvement of some hybrids which could be attributed to genetic mixing. Futhermore, significant differences ($P < 0.05$) in terms of tocopherol content were observed amongst hybrids with the same mother-clone. One of the main objectives of nutraceutical industries is to identify plant matrices rich in antioxidant compounds, for the preparation of new phytoderived products. Some hybrids studied like SNK13 × T79/501, SNK413 × SCA12, SCA12 × ICS40 and SCA12 × SNK13 seem to be the good candidates for these industries. Nevertheless, chromatographic analysis does not reveal the presence of γ-tocotrienols (**Figure 3**). An exploration of other forms of tocotrienols in cocoa beans should be interesting.

3.4. Principal Component Analysis (PCA)

A PCA was performed using the content of tocopherols obtained after chromatographic analysis. A biplot of the first two principal components (PC1 = 60.12% and PC2 = 37.37%) is shown in **Figure 4** for fermented beans. SNK13 × T79/501 was located at the negative axis of F1 and was correlated positively to β and δ-tocopherol while SNK413 × SCA12 and SNK413 × T79/501 were located at the positive axis of F2 and correlated to γ-tocopherol. A PCA biplot of fatty acids (PC1 = 54.61% and PC2 = 25.59%) is shown in **Figure 5**. Here, the clustering depicted the predominance of fatty acids (palmitic, oleic, palmitoleic, linoleic) in

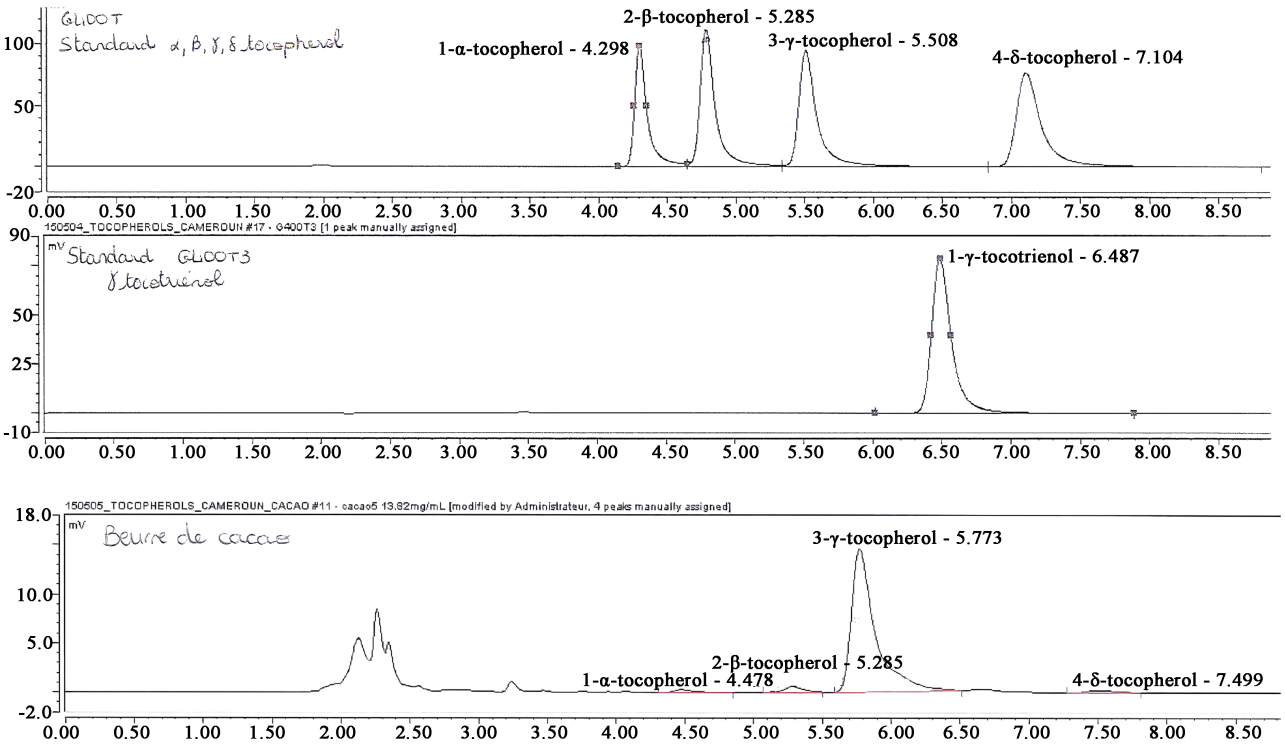

Figure 3. Chromatograms for evaluation of tocopherols and tocotrienols by UHPLC.

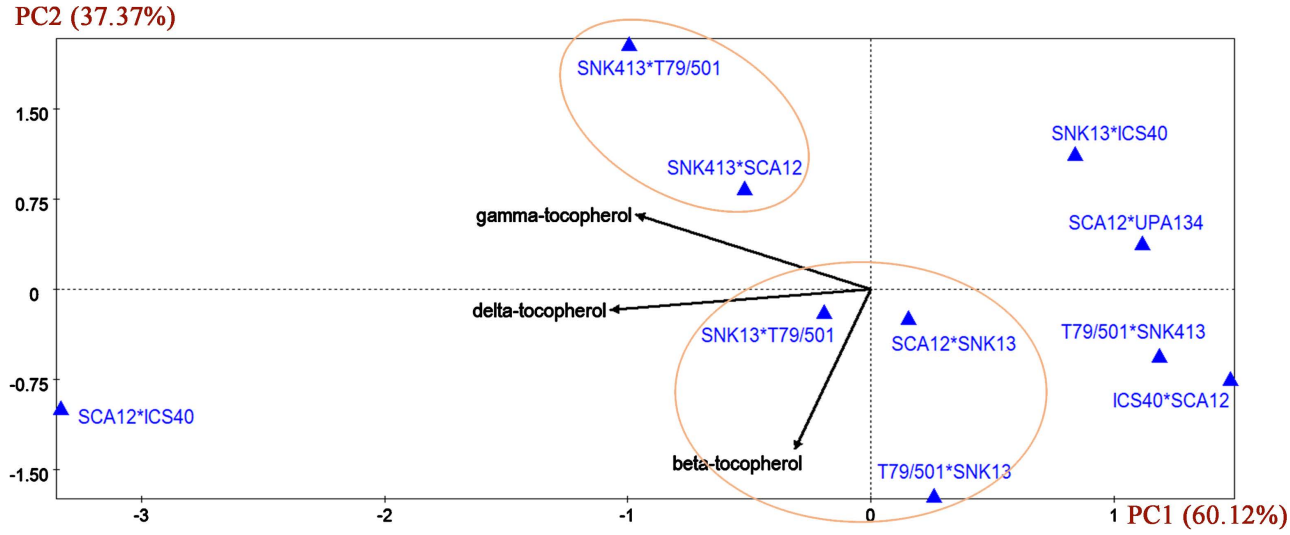

Figure 4. Principal Component Analysis (PCA) bi-plot of the tocopherols detected in fermented cocoa beans.

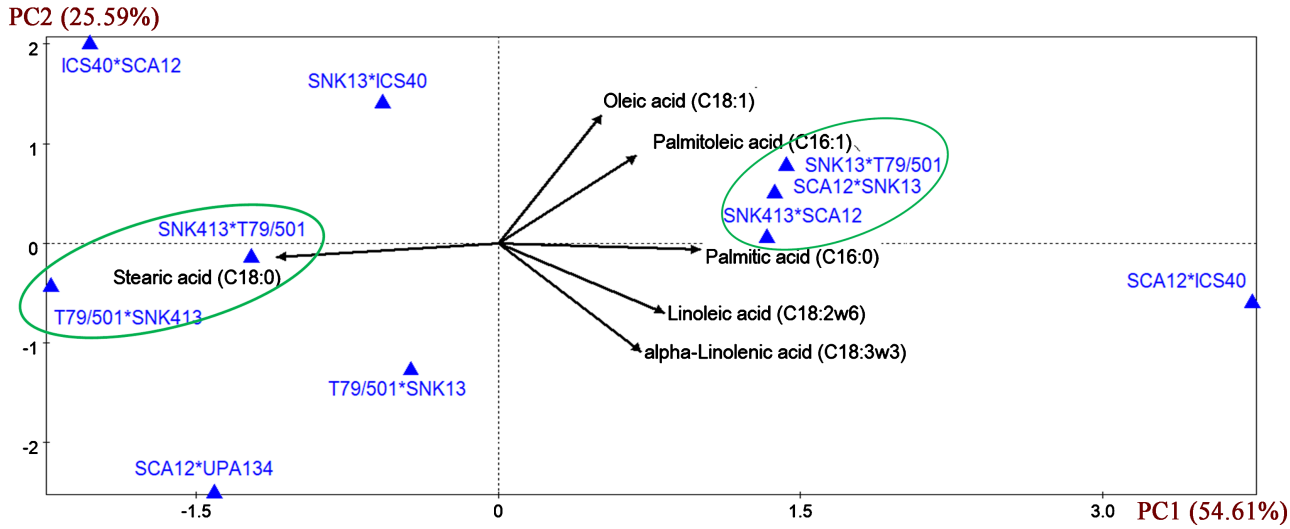

Figure 5. Principal Component Analysis (PCA) bi-plot of the fatty acids detected in fermented cocoa beans.

three hybrids namely SNK13 × T79/501, SCA12 × SNK13 and SNK413 × SCA12, located at the postive axis of F2. Contrarily, the hybrids T79/501 × SNK13, SCA12 × UPA134 and T79/501 × SNK413 were located at the negative axis of F1. These results indicate that the fatty acid and tocopherol profile can be used to discriminate some hybrids. However, the impact of fermentation on this profile should not be neglected. The genetic origin of each hybrid could also be a major factor in the biosynthesis of these compounds. In fact, several authors have found that genotypes and post-harvest practices may affect the chemical composition of cocoa beans [15] [19] [42].

4. Conclusion

In this work, we report for the first time the fatty acid composition, tocotrienol and tocopherol profiles of some hand-pollinated cocoa hybrids from Cameroon.

Information on the impact of the genotype on the cocoa fat composition was provided. SNK413 × SCA12 and T79/501 × SNK13 are both hybrids with highest commercial value based on their TF content. SCA12 × ICS40, SCA12 × SNK13, SNK13 × T79/501 and SNK413 × SCA12 which recorded the highest levels of UFA, have the least hard cocoa butters. No γ-tocotrienol was found in fermented cocoa samples. Nonetheless, some studied hybrids seem to be the good candidates for nutraceutical industries. A differentiation in terms of total fat and tocopherol content was observed amongst hybrids with the same mother-clone, suggesting an impact of pollen on these compounds. Crosses with SCA12 (Forastero) and SNK (Trinitario) clones seem to be very interesting. Therefore, local breeding programs should intensify research towards these clones although the climate changes have not been considered in this study.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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