Original Research Article

Evaluation of Phenolics and Aromatics Compounds of Placenta Three Cocoa Varieties (*Theobroma cacao L*) Cvs ‘ultivated in Cote d Ivoire'

ABSTRACT

Cocoa placenta obtained after the removal of beans is a huge cocoa by-product usually regarded as waste by farmers. Cocoa placenta obtained from three cocoa cultivars, including forastero, criollo and national were investigated for the first. This study was undertaken to provide a more evaluate comprehensive characterization of aromatics and phenolics in cocoa placenta. For this, 3 cocoa placenta widely produced in Côte d’Ivoire were selected and oven-dried (50°C/3 days) after fermentation before HPLC-MS/MS and SPME-GC-MS analysis of phenolics and aromatics compounds. A total of 14 phenolic compounds were identified in all cocoa placenta samples with the highest amount observed in criollo (212.99 mg/g). Kaempferol pentosyldihexoside was found as the major phenolic substance in all the samples with an important amount in criollo (54.63 mg/g). According to the findings of compounds volatiles profile , a total of 48, 55 and 60 aroma constituents with variable groups including esters, alcohols, aldehydes, terpenes, furans, acids, volatile phenols, hydrocarbons, and ketones were detected in criollo, forastero and national, respectively. The highest total amount was calculated in national placenta (406.76 mg/kg). 2-hexanol (73.56 mg/kg), 3-Methyl-3-pentanol (19.96 mg/kg), Methoxy-1-butanol (11.75 mg/kg) was the prominent volatile substance in all cocoa placenta. The results of the present study clearly demonstrate that cocoa placenta is a potential by-product that need to be used in animal feed or human food formulation order to be valued in the dietetic field.

Keywords: *Cocoa placenta; phenolics; aromatics; profile*

1. INTRODUCTION

Cocoa (Theobroma cacao) is probably originated from central America and have been widely cultivated in some parts of the globe. Africa is the leader of cocoa beans production, covering 73%, followed by America 17%, Asia and Oceania 9,9% [1]. Cote d ivoire, Ghana and Indonesia covered 67% globally. Besides cocoa beans, mostly composed of nibs, cocoa

pods produce several residuals by products anually, including cocoa pod husk, cocoa bean shell, cocoa sweating or juice and cocoa placenta [2]. It has been reported that when 1 tonne of cacao beans is produced, ten tonnes of cacao by products are generated and less exploited, which are discarded as wastes causing serious environmental issues in cocoa farms and cocoa beans processing platforms. Their disposal is generally carried out without an adequate treatment, resulting in cocoa plant deseases like black pod rot bad odor, cocoa farmers health concerns [2,3]. The wastes by products are rich in organic matter, including phenolic compounds, aromatics and inorganic elements [4]. In recent year, high added–value products from cocoa waste by product such as biogas,poultry and livestock feed, fertilizers, potassium carbonate, nutraceutical products and other chemical products have gained increasing interrest for their reduction and potential applications in agriculture, cosmetic, food and beverage, pharmaceutical and fine chemical industries [2,5].
Indeed, these compounds may help to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke, arthritis, diabetes and inflammatory bowel diseases [6,7]. Among these groups, phenolics and aromatics compounds are important for to human health and well-being [6,8].
Cocoa placenta, one of the four main generated cocoa waste by product is regarded as the most underutilize cocoa by products. In fact, when the cocoa beans fermentation is done, cacao placenta are separated from the beans before drying. After separation, cocoa placenta are abandoned and left to dry on farms without their use for added value products, which can result to environmental problems [9]. The cocoa placenta among the by products, are also rich in organic matter, phytochemicals compounds and inorganic elements [10]. Therefors a solution to this environmental concerns could be to implement valorisation methods for cocoa by products and mainly cacao placenta. For instance, cocoa placenta could be used for the development of human and animal dietary supplement, functional food, food additive and animal feed, cosmetic and pharmaceutical products. Futhermore, the new policy to the revaluation of cocoa residues may become considerable for the sustanability of the economy in a country like Côte d'Ivoire, which is considered to be the leader of cocoa cultivation and production worldwide [1].
In this country, three maın cocoa tree have been cultivated, including forastero, criollo and mercedes (national). Cocoa industry in Cote d Ivoire has a huge social revelance since cocoa is the first most important cultivated crops nationalwide and the core of Ivorian economy. However, for our best knowledge no study has yet been publish on the phenolics compounds and aroma profiles of cacao placenta in literature.
Therefore the aim of the present study was first, to elucidate the phenolics compounds and aroma profiles of cocoa placenta from three cocoa varieties (Forastero, criollo and national) in order to valorize cocoa placenta for its potential use in animals feed and humans food formulation.

2. MATERIAL AND METHODS

2.1 Plant Material Collection and Preparation

Cocoa placenta were collected from three cocoa varieties ripe pods (Forastero, criollo and national) in cocoa farms of Lodjiboua region/ Côte d Ivoire in september 2021. Forastero, Criollo and national cocoa pods were differently havested with hand and perch. The havested cocoa pods were differently grouped and cut with knife to remove altogether the beans and the placenta from the pods husk. Thereafter, the beans and the placenta together were naturally fermented on the banana leaves for 5 days. Then, the placenta were separated from the beans mass and differently packed into labaled humid proof plastic bags and immediatly sent to Biochemical laboratory of technical high school of Yopougon for drying.

Once at the laboratory, the naturally fermented fresh placenta from the three cocoa cultivars were weight and dried in a convective dryer (UM 400, Memmert GmbH, Germany) set at 50°C for 48 hours (moisture content <8%). The oven dried cocoa placenta were crushed into fine powder by the means of lab grinder model. The milled powder obtained were sieved using a 250 um mesh sieve. The final sieve powders were packed and sealed into labaled humid proof plastics bottles and stored at room temperature 20-25 until use.

2.2 Chemical

The Distilled water used during this study was purified through a Millipore-Q system (Milliport Billerica, Massachussets, USA). Sodium hydroxide, sulfuric acid, sodium carbonate, chlorhydric acid, Folin Ciocalteu were purchased from Sigma Co (St. Louis, MO). potassium persulfate, ethanol and methanol were obtained from Merck (Gernsheim, Germany). All aroma and phenolic references were purchased from Sigma Co (St. Louis, MO). All reagents and solvent used in this work were analytical grade when necessary.

2.3 The Total Phenolics Content (TPC)

Polyphenols content was determined using the method reported by [11]**.** A quantity (1g) of dried powdered sample was soaked in 10 mL of methanol 70% (w/v) and centrifuged at 1000 rpm for 10 min. an aliquot (1 mL) of supernatant was oxidized with 1 mL of folin-Ciocalteu's reagent and neutralized by 1 mL of 20% (w/v) sodium carbonate. The reaction mixture was incubated for 30 min at ambient temperature and absorbance was measured at 745 nm by using a spectrophotometer (PG Instruments, England). The polyphenols content was obtained using a calibration curve of gallic acid (1 mg/mL) as standard.

2.4 HPLC-DAD–ESI-MS/MS Analysis of Phenolic Compounds

Phenolic compounds analysis was firstly performed based on an extraction technique described by [12] with slight modification. Briefly, 1.0 g of each cocoa placenta powder sample was put into a centrifuge tube and 10 mL of methanol/water (80 20 v/v) mixture was added and stirred for one minute in a vortex apparatus. Thereafter, the tubes were centrifuged at 4,800 g for 10 min at 4°C. The extraction was repeated twice. The methanol in each collected supernatant was slightly evaporated at 40°C. Then, the extracts were filtered through 0.45 µm membrane filters (Whatman Inc., Clinton, NJ).
HPLC-MS/MS (Agilent 1260 HPLC system; Agilent Technologies, Palo Alto, California, USA) analysis. The HPLC equipped with a diode array detector (DAD) was used. The HPLC-MS/MS (LC-MS/MS, Shimadzu LC 8040) system equipped with a diode array detector (DAD) was consisted of electro spray ionization (ESI), two pumps (LC-30 AD), a column oven (CTO-10AD VP), an autosampler (SIL-30 AC) and a degassing unit (DGU-20A 3R). The MS/MS system was operated at 300 capillary temperature, 350 vaporizer temperature, nebulizer pressure, 30 psi, nebulizer gas flow of 1.25 L·min−1 2500 V spray voltage (negative polarity), 4000 V spray voltage (positive polarity). Samples and references were filtered before injecting 20 µl into a C18 reversed-phase column (ODS hypersil 4.6 mm x 250 mm, 5 um). The column was programmed at 30 C, and analysis was conducted for 34 min. The mobile phase consisted of two solvents: Solvent A, water /formic acid (99:1; v/v) and Solvent B, HPLC grade methanol. The flow rate was adjusted to 0.7 mL·min−1 with a constant temperature of 30°C, and the following gradient solution was applied: 0 min, 100% A; 1 min, 100% A; 25 min 5% A and 9% B; 25 min, 5% A and 95% B; 30 min, 0% A and 100% B. Each phenolic compound was identified and assigned by the comparison of retention time and UV spectra to pure external standards prepared with methanol at concentration of 0, 50, 75, 100, 150, 200 ppm. Individual compounds were quantified using a calibration curve of the corresponding reference compounds [12,13].

2.5 Extraction and GC-FID/GC-MS Analysis of Volatile Compounds

Aroma compounds of cocoa placenta samples were analysed using SPME-GC-MS. The volatiles were extracted by solid-phase micro-extraction techniques described by [14] using a multipurpose sampler (Gerstel, Mülheim and der Rur, Germany) equipped a HS-SPME unit. Briefly, 2 g of cocoa placenta powder was placed into 20 mL vial and 1 µl of internal standard solution nonane with a concentration of 24,5 ug/µl. The vial was hermetically sealed and preincubated at 40 for 30 min in water bath. Thereafter, a well-conditioned SPME fiber (2 cm, 50/30 mm stableFlex fiber divinylbenzene/carboxen on polydimethylsiloxane (DVB/CAR/PDMS SPME) (Supelco, sigma-Aldrich, Belgium) was exposed to the head space analysis at 40°C for 45 min at 500 rpm. The volatiles extraction was performed in three repetitions.
After the isolation of volatiles constituents, the fiber was placed in a gas chromatography system (GC, Shimadzu GC-2010 Plus capillary, scientific instrument, Japan). The analysis was conducted using splitless injection mode, and the injection port was set at 250 for desorption, while the FID was programed at 260°C. The GC program was applied as follows: Kept at 40°C (10 min), from 40 to 180°C (5/min) and held for 1 min, then increased to 250°C (10/min) and held for 1 min. The resulting peaks corresponding to volatiles compounds were identified using mass spectral database Wiley (Wiley 275) **(McLafferty, 2005**), FFNSC (Flavor and Fragrance of Natural and Synthetic compounds), retention index, GC's internal library and chemical references. The concentration of determined aroma compounds were calculated based on internal standard method and peak area. Retention indices of the compounds were determined using the retention time of n-alkane (C7−C30) series [16]**.**

2.6 Statistical Analysis

All analyses were carried out in triplicates and data expressed as means ± standard deviation. One way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess significant differences between means ($p<0.05$) using XLStat 2017 software

3. RESULTS AND DISCUSSION

3.1 Total Phenolics and Phenolics Profile

Phenolic compounds identified in the cocoa placenta samples are presented in table 1. A total of 14 phenolic constituents were detected and quantified in all cocoa placenta samples including gallic acid, catechin, chlorogenic acid, caffeic acid, epicatechin, syringic acid, p-coumaric acid, quercetin-3-glucoside, theobromine, quercetin hexoside, 3-caffeoylquinic acid, di-caffeoylquinic acid, and Kaempferol pentosyldihexoside. Similar phenolic compounds were detected with a variable amount in all cocoa placenta samples. The total quantities of these substances were significantly different ($p<0.05$), and 212.99, 146.20 and 109.01 g/kg were determined in criollo, national and forastero cocoa placenta, respectively. Phenolic constituents are important as they contain high antioxidant activity and prevent food oxidation process. Gallic acid belonging to hydroxybenzo was only detected in low quantities in 3 placenta as indicated in the Table 1. It's important recalling that gallic acid is known to possess antimicrobial activity against human pathogens and this molecule can also scavenge electrophilic mutagens [17]. This low quantities of gallic acid may be due to the fact that hydroxybenzoic acids contents of edible plants are generally low contrary to hydroxycinnamic acids (p-coumaric, caffeic, ferulic, chlorogenic and sinapic acids) [18]. Considering Kaempferol pentosyldihexoside, Di-caffeoylquinic acid, caffeoylquinic acid and chlorogenic acid, these compounds were detected and quantified in cocoa placenta for the first time in this present study. However, the amounts of these substances were probably

decreased owing to the heat treatment applied during drying process. These statements were reported by some authors in fruits and vegetables (**Alvarado-Ramirez et al., 2018**). Of all phenolic compounds identified in cocoa placenta, Kaempferol pentosyldihexoside (54.63 g/kg) and Di-caffeoylquinic acid (33.78 g/kg) were quantitatively the most abundant phenolic compounds in the placenta of criollo, follow by caffeoylquinic acid (20.36 g/kg) in the placenta of forastero and chlorogenic acid (18.41 g/kg) in the placenta of national. The highest amount of phenolic compounds was observed in criollo placenta samples. Studies made by [20] and [21] have shown thatextrinsic factors (geographical and climatic), genetic factors, but also the degree of plant maturation and storage time have astrong influence on polyphenol content.Based on anti-inflammatory and antioxidant activities of chlorogenic acid, consumption of placenta could help prevent diseases such as diabetes, obesity and cancer [22]. Thus, the utilization of cocoa placenta as value added products for animal feed formulation could considerably reduce their discharged in the cocoa farms and environment pollution.

Table 1: Phenolics composition of cocoa's placenta samples

		Concentration (g/kg) ±SD		
RT	Phenolic compounds	Forastero	Criollo	National
3.64	Gallic acid	8.0 ± 3.4^{a}	2.6 ± 0.9^{c}	4.4 ± 0.5^{b}
12.39	Catechin	5.9 ± 1.2^{b}	12.4 ± 4.2^{a}	3.9 ± 1.4^{c}
14.96	Chlorogenic acid	2.7 ± 0.3^{c}	7.0 ± 2.2^{b}	18.4 ± 6.3^{a}
18.10	Caffeic acid	7.3 ± 2.1^{b}	13.7 ± 4.1^{a}	11.2 ± 2.9^{a}
22.76	Epicatechin	5.5 ± 0.9^{c}	17.6 ± 2.4^{a}	9.3 ± 1.7^{b}
27.90	Syringic acid	2.5 ± 0.4^{c}	13.4 ± 3.6^{a}	6.2 ± 1.7^{b}
32.85	P-coumaric acid	7.3 ± 2.5^{b}	10.1 ± 2.8^{a}	8.6 ± 0.9^{b}
39.11	Quercetin-3-glucoside	2.2 ± 0.6^{b}	10.0 ± 1.4^{a}	1.5 ± 0.8
34.78	Theobromine	6.5 ± 1.5^{b}	16.8 ± 4.2^{a}	2.9 ± 0.6^{c}
42.74	Quercetin hexoside	8.7 ± 3.4^{b}	11.6 ± 4.5^{a}	6.5 ± 1.2^{c}
18.54	3-caffeoylquinic acid	20.4 ± 6.1^{a}	9.4 ± 2.3^{c}	16.7 ± 6.8^{b}
21.33	Di-caffeoylquinic acid	14.2 ± 2.4^{c}	33.8 ± 11.7^{a}	18.6 ± 7.4^{b}
43.89	Kaempferol pentosyldihexoside	17.9 ± 4.6^{c}	54.6 ± 9.8^{a}	38.0 ± 7.2^{b}
	Total phenolic compounds	**109.01**	**212.99**	**146.2**

Values are means and standard deviations (mean (SD)) of three measurements for color analysis and three replications for other evaluations. Different lowercase letters on the numbers in the same line represent significant differences (p <0.05).

3.2 Volatile Constituents and Volatile Profile

The composition of volatile components and amounts characterized in the cocoa placenta samples are displayed in Table 2, while the difference between the cocoa placenta samples and the aroma classes are displayed in the Figure 1. Based on the GC-MS results, a total of 48, 55 and 60 aroma constituents with variable groups including esters, alcohols, aldehydes, terpenes, furans, acids, volatile phenols, hydrocarbons, and ketones were detected in criollo, forastero and national, respectively. No previous information related to the aroma substances of cocoa placenta of the three varieties of cocoa placenta samples investigated in this present study was reported. The major aroma group was alcohols in all the cocoa placenta samples followed by aldehydes, acids, furans and ketones (Table 2). The total concentration of the volatile substances in national cocoa placenta samples (406.76 mg/kg)

was significantly higher than those of forastero (314.74 mg/kg) and criollo (219.92 mg/kg) samples. Interestingly, alcohols were qualitatively and quantitatively the most abundant volatile groups accounting for 156.55, 81.81, and 80.09 mg/kg, in national, forastero and criollo, respectively. These amounts represented 38.45, 36.41, and 25.9% of the overall aroma compounds in national, criollo and forastero cocoa placenta samples, respectively. It is well established that these compounds are formed from unsaturated fatty acids via the lipoxygenase activity mainly when cell structure is disintegrated in oxygenated environment. Moreover, 2-Hexanol was found to be the most dominant volatiles in all the samples (Table 2). Generally, sweety, fresh, oily, herbal, tropical fruits, floral, and alcohol notes are attributed to volatile alcohols [22].

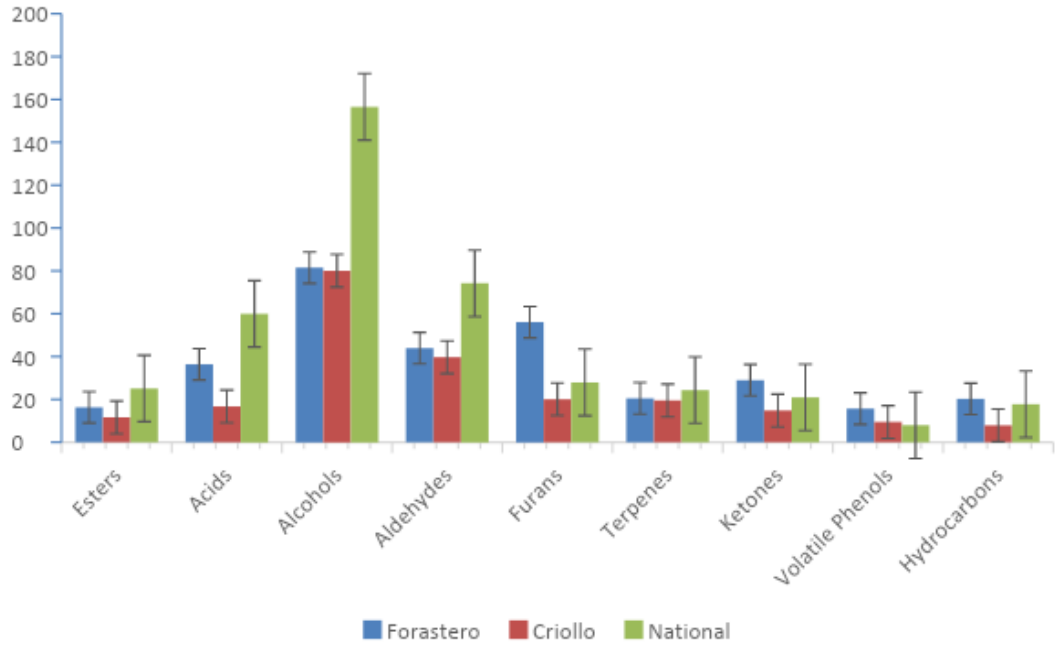

Figure 1: Main group of aroma components detected and their content in each sample of cocoa's placenta

In the present study, esters (7), alcohols (16), acids (7), aldehydes (8), volatiles phenols (3), furans (4), hydrocarbons (3), terpenes (6), ketones (5) were detected in national cocoa placenta samples. Aldehydes (74.19 mg/kg) were the second abundant group of volatiles component in national cocoa placenta samples, follow by acids as the third dominant class of volatiles substances in the samples. In addition to 2-Hexanol, Acetic acid (18.33 mg/kg) and 2-Methyl-2-butanol (17.9 mg/kg) were determined in large quantity in the samples. These two compounds were more prominent in national cocoa placenta samples than forastero and criollo. Acids are mostly converted into aldehydes, alcohols and lactones during thermal process (références). This class of aroma substances with often green, sweety, vinegar, floral notes rarely contribute to the odor of food matrix owing to their high threshold values.

As of the forastero cocoa placenta samples, esters (6), aldehydes (11), alcohols (12), furans (5), ketones (5), terpenes (3), acids (6), volatiles phenols (5), hydrocarbons (3) were determined as the main volatile constituents (Table 2). Among the volatiles detected in forastero placenta, 3-Methyl-3-pentanol was calculated as the second largest aroma compound. When compared to national and criollo, furans were found in large amount in forastero cocoa placenta samples, in which 5-Hydroxymethylfuran was found as the

prominent furans in forastero placenta samples. This O-heterocyclic class of compound is the product of drying and roasting processes, and significantly contribute to caramel-like, burnt, roasted, sweety, and nutty odor notes with slightly lower threshold values (références). In this study, most of the detected furans are the results of the drying process applied.
In criollo placenta samples, the volatile constituents were determined to be esters (4), acids (6), alcohols (11), aldehydes (8), furans (5), volatile phenols (3), ketones (4), terpenes (5), and hydrocarbons (2). In addition to alcohols, furans were quantitatively detected as the second class of aroma in criollo placenta samples. Of all the volatiles constituents quantified in criollo placenta samples, 2-phenylethanol was the most predominant volatile compound, follow by 3-Methoxy-1-butanol (Table 2). When the aroma numbers and the total volatile amounts of samples were compared, criollo placenta samples were found to contain the lowest volatiles and total aroma amounts. These substantial differences could probably result from divers factors, such as growing pratices, microorganisms activities during fermentation, beans physical and chemical compositions (référence).

Table 2: Volitile composition of cocoa's placenta samples

		Concentration (mg/Kg) ± SD			
Compounds	LRI	Forastero	Criollo	National	Identification
Esters					
Ethyl acetate	577	1.4 ± 0.1[a]	<LOD	0.5 ± 0.2[a]	LRI, MS, tent
Ethylbutyrate	1044	<LOD	<LOD	6.2 ± 1.3	LRI, MS, tent
Ethyl-2-methylbutyrate	1052	6.3 ± 2.1[a]	2.4 ± 0.8[b]	1.9 ± 0.2[b]	LRI, MS, tent
Ethyl-3-methylbutyrate	1068	3.78 ± 0.4[a]	<LOD	0.6 ± 0.1[b]	LRI, MS, tent
Isoamyl acetate	1125	4.23 ± 0.2[b]	3.0 ± 0.7[b]	7.9 ± 2.4[a]	LRI, MS, tent
2-Phenylethyl acetate	1815	0.5 ± 0.1[c]	5.6 ± 1.2[a]	3.6 ± 0.9[b]	LRI, MS, tent
Isobutyl acetate	1012	0.2 ± 0.1[b]	0.7 ± 0.3[b]	4.5 ± 1.3[a]	LRI, MS, tent
		16.3	**11.64**	**25.1**	
Acids					
Acetic acid	1400	9.6 ± 2.4[b]	5.8 ± 1.3[c]	18.3 ± 4.7[a]	LRI, MS, tent
Hexanoic acid	1807	3.7 ± 0.8[b]	1.7 ± 0.4[c]	8.5 ± 1.6[a]	LRI, MS, tent
Nonanoic acid	2124	<LOD	2.3 ± 0.6[b]	12.9 ± 3.1[a]	LRI, MS, tent
Heptanoic acid	1960	7.9 ± 2.4[a]	4.4 ± 1.2[b]	4.7 ± 0.9[b]	LRI, MS, tent
Decanoic acid	2313	2.5 ± 0.2[a]	0.9 ± 0.2[b]	2.2 ± 0.4[a]	LRI, MS, tent
Octanoic acid	2041	11.4 ± 3.2[a]	1.7 ± 0.1[c]	8.5 ± 3.2[b]	LRI, MS, tent
2-Ethyl hexanoic acid	1911	1.4 ± 0.2[b]	<LOD	4.8 ± 1.3[a]	LRI, MS, tent
		36.39	**16.72**	**59.94**	
Alcohols					
2-Methyl-2-Butanol	1003	5.9 ± 1.5[c]	7.7 ± 2.9[b]	17.9 ± 4.8[a]	LRI, MS, tent
2-Methyl-2-pentanol	716	2.6 ± 0.8[b]	<LOD	4.4 ± 1.3[a]	LRI, MS, tent
3-Methyl-3-pentanol	1080	20.0 ± 6.4[a]	10.9 ± 2.9[b]	5.3 ± 0.9[c]	LRI, MS, tent
3-Methoxy-1-butanol	790	4.1 ± 1.3[c]	11.8 ± 3.6[a]	8.6 ± 2.2[b]	LRI, MS, tent
2-Hexanol	1254	8.9 ± 3.4[b]	7.0 ± 1.4[b]	73.6 ± 11.3[a]	LRI, MS, tent
Phenethyl alcohol	1859	6.5 ± 2.1[a]	1.9 ± 0.3[c]	3.9 ± 0.7[b]	LRI, MS, tent
2-phenylethanol	1867	9.9 ± 3.2[c]	16.7 ± 4.1[a]	12.7 ± 3.2[b]	LRI, MS, tent
2-methyl-3-octanol	2006	<LOD	<LOD	5.7 ± 1.3	LRI, MS, tent
1,3-Butanediol	1578	3.9 ± 0.6[b]	6.8 ± 1.7[a]	1.8 ± 0.1[c]	LRI, MS, tent

2,3-Butanediol	1548	9.7 ± 2.1[a]	2.9 ± 0.8[c]	6.7 ± 2.3[b]	LRI, MS, tent
1-Octanol	1546	<LOD	3.4 ± 0.7[a]	0.9 ± 0.1[b]	LRI, MS, tent
3-Octanol	1396	<LOD	<LOD	4.7 ± 0.9	LRI, MS, tent
Isopropyl alcohol	920	<LOD	<LOD	1.9 ± 0.3	LRI, MS, tent
Lauryl alcohol	1981	2.3 ± 0.9[b]	4.2 ± 0.8[a]	0.5 ± 0.2[c]	LRI, MS, tent
2-methyl-3-octanol	1096	2.7 ± 0.5[b]	6.9 ± 1.2[a]	1.0 ± 0.3[c]	LRI, MS, tent
Isoamyl alcohol	1220	5.0 ± 1.4[b]	<LOD	7.1 ± 3.2[a]	LRI, MS, tent
		81.51	**80.09**	**156.55**	
Aldehydes					
Hexanal	1078	11.3 ± 2.7[a]	2.7 ± 0.8[c]	6.9 ± 1.3[b]	LRI, MS, tent
Ethylbenzaldehyde	1730	0.9 ± 0.1[c]	5.6 ± 1.4[a]	2.4 ± 0.5[b]	LRI, MS, tent
(*Z*)-2-Heptenal	1319	0.3 ± 0.1[c]	2.4 ± 0.4[b]	5.3 ± 1.3[a]	LRI, MS, tent
(*E*)-2-Heptenal	1334	2.4 ± 0.7[c]	5.7 ± 2.4[b]	15.7 ± 3.4[a]	LRI, MS, tent
Isovaleraldehyde	916	0.26 ± 0.1[b]	3.9 ± 0.2[a]	<LOD	LRI, MS, tent
Benzaldehyde	1508	0.2 ± 0.1[b]	<LOD	2.0 ± 0.3[a]	LRI, MS, tent
(*Z*)-3-Hexenal	1137	4.9 ± 1.2[b]	<LOD	12.3 ± 4.1[a]	LRI, MS, tent
Piperonal	2216	6.4 ± 1.7[a]	2.2 ± 0.4[b]	<LOD	LRI, MS, tent
(*E*)-2Hexenal	1219	7.5 ± 2.6[c]	9.2 ± 3.9[b]	14.6 ± 3.2[a]	LRI, MS, tent
5-Methyl-2-furfural	1575	3.8 ± 0.8[c]	7.9 ± 1.2[b]	11.4 ± 2.7[a]	LRI, MS, tent
Furfural	1469	6.0 ± 1.1[a]	ND	3.6 ± 0.4[b]	
		43.92	**39.71**	**74.19**	
Volatile phenols					
Phenol	1958	8.0 ± 2.4[a]	4.4 ± 1.4[b]	1.5 ± 0.4[c]	LRI, MS, tent
2,5-Dimethylphenol	2068	2.4 ± 0.6[a]	3.44 ± 0.9[a]	<LOD	LRI, MS, tent
P-Cresol	2074	5.3 ± 1.2[a]	<LOD	4.3 ± 1.3[a]	LRI, MS, tent
3-Ethylphenol	2150	<LOD	1.5 ± 0.3[b]	2.2 ± 0.7[a]	LRI, MS, tent
		15.71	**9.47**	**7.96**	
Furans					
2-Acetylfuran	1536	11.4 ± 2.1[a]	7.7 ± 2.6[b]	<LOD	LRI, MS, tent
2-Acetyl-5-methylfuran	1608	9.2 ± 3.4[b]	0.4 ± 0.1[c]	11.8 ± 2.8[a]	LRI, MS, tent
5-Hydroxymethylfuran	2395	18.4 ± 6.2[a]	4.7 ± 1.3[b]	2.2 ± 0.9[c]	LRI, MS, tent
2,5-Dimethylfuran	958	2.2 ± 0.4[c]	5.3 ± 1.6[b]	9.6 ± 2.4[a]	LRI, MS, tent
2-Pentylfuran	1238	14.8 ± 4.5[a]	2.0 ± 0.7[c]	4.2 ± 0.3[b]	LRI, MS, tent
		56.04	**20.07**	**27.93**	
Hydrocarbons					
P-Xylene	1130	8.4 ± 2.6[b]	2.7 ± 0.4[c]	11.6 ± 3.2[a]	LRI, MS, tent
O-Xylene	1169	2.6 ± 0.6[a]	<LOD	3.2 ± 0.3[a]	LRI, MS, tent
Styrene	1245	9.3 ± 2.5	5.1 ± 1.2	2.9 ± 0.8	LRI, MS, tent
		20.26	**7.88**	**17.78**	
Terpenes					
dl-Limonene	1206	9.5 ± 2.4	0.8 ± 0.2	1.8 ± 0.4	LRI, MS, tent
Linalool	1565	<LOD	6.6 ± 1.4	<LOD	LRI, MS, tent
1,8-Cineol	1209	4.8 ± 1.3[a]	<LOD	0.9 ± 0.4[b]	LRI, MS, tent

β-Phellandrene	1245	1.4 ± 0.8[c]	3.3 ± 0.4[b]	11.7 ± 4.3[a]	LRI, MS, tent
Linaloxide	1448	<LOD	<LOD	3.9 ± 1.1	LRI, MS, tent
P-Cymene	1268	<LOD	2.4 ± 0.5[b]	5.6 ± 1.7[a]	LRI, MS, tent
Sabinene	1128	4.9 ± 0.8[b]	6.5 ± 2.3[a]	0.4 ± 0.1[c]	LRI, MS, tent
		20.5	**19.52**	**24.32**	
Ketones					
2,3-Butanedione	977	<LOD	0.8 ± 0.3[b]	2.9 ± 0.6[a]	LRI, MS, tent
Bezophenone	2470	1.5 ± 0.3[b]	5.6 ± 1.3[a]	1.0 ± 0.3[b]	LRI, MS, tent
4-Methyl-2-pentanone	1028	6.6 ± 2.4[b]	<LOD	9.2 ± 1.5[a]	LRI, MS, tent
2-octanone		4.7 ± 0.6	<LOD	<LOD	LRI, MS, tent
3-Hydroxy-butanone	1291	0.9 ± 0.4[b]	5.6 ± 0.9[a]	1.3 ± 0.4[b]	LRI, MS, tent
2-(*5H*)-furanone	1761	10.5 ± 3.2[a]	2.9 ± 0.4[c]	6.6 ± 2.6[b]	LRI, MS, tent
		28.96	**14.82**	**20.95**	
Grand Total		**314.74**	**219.92**	**406.76**	

** LOD: limit of detection; LRI: linear retention index computed on DB-WAX capillary column; MS tent (tentatively identified by MS): when MS or LRI is used for the identification of a constituent, it must be stated as an attempt of identification. The data are shown as means and standard deviations (mean (SD)) of three determinations. Different lowercase letters on numbers in the same line represent significant difference ($p < 0.05$).*

4. Conclusion

The present study was conducted to elucidate the phenolic compounds and volatile constituents of cocoa placenta obtained from three different cocoa cultivars, including forastero, criollo and national. Phenolic compounds were identified through HPLC-MS/MS, while the aroma substances were isolated using SPME method and determined via GC-MS for the first time. Based on the results obtained, cocoa placenta possesses a substantial amount of phenolic compounds. The highest amount of phenolics was determined in criollo. The aroma substances were more intense in national cocoa placenta sample. Alcohols and aldehydes were found to be predominant class of aroma in all cocoa placenta samples. 2-hexanol was also detected as the dominant aroma constituent in all the samples. According to the findings collected in this study, cocoa placenta could be a potential candidate for animal feed and a good source of bioactive substance to be extracted for human food formulation in order to mitigate its disposal in the environment as waste.

Competing interests

Declaration of competing interest should be placed here. All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. If no such declaration has been made by the authors, SDI reserves to assume and write this sentence: “Authors have declared that no competing interests exist.”.

References

1. ICCO. Quarterly Bulletin of Cocoa Statistics, 2017, https://www.icco.org/about-us/icco-news/384-february2018-quarterly-bulletin-of-cocoa-statistics.html.
2. Vásquez ZS, de Carvalho Neto DP, Pereira GV, Vandenberghe LP, de Oliveira PZ, Tiburcio P B, Soccol CR. Biotechnological approaches for cocoa waste management: A review. Waste Management,2019; 90, 72-83.
3. Cádiz-Gurrea MdlL, Fernández-Ochoa Á, Leyva-Jiménez FJ, Guerrero-Muñoz N, Villegas Aguilar MdC, Pimentel-Moral S, Segura-Carretero A. LC-MS and spectrophotometric approaches for evaluation of bioactive compounds from Peru cocoa by-products for commercial applications. Molecules2020;, 25(14), 3177.
4. Sobamiwa O, Longe O. Utilization of cocoa-pod pericarp fractions in broiler chick diets. Animal Feed Science and Technology. 1994 ; 47(3-4), 237-244.
5. Kone K. Akueson K. Norval G. On the production of potassium carbonate from cocoa pod husks. Recycling 2020; 5(3), 23.
6. Liu RH. Health-Promoting Components of Fruits and Vegetables in the Diet. Adv Nutr An Int Rev J. 2013;4(3):384S–392S.
7. Andarwulan N, Kurniasih D, Apriady RA, Rahmat H, Roto A V., Bolling BW. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. J Funct Foods. 2012;4(1):339–47.
8. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr. 2004; 134(12 Suppl):3479S–3485S.[Cited 2017 Sep].
9. Martínez R, Torres P, Meneses M, Figueroa J, Pérez-Álvarez J, Viuda-Martos M. Chemical, technological and in vitro antioxidant properties of cocoa (Theobroma cacao L.) co-products. Food Research International,2012; 49(1), 39-45.
10. Goude KA, Adingra KMD, Gbotognon OJ, Kouadio E. (2019). Biochemical characterization, nutritional and antioxidant potentials of cocoa placenta (Theobroma Cacao L.). Annals Food Science and Technology, 2019 ;20(3), 603-613.
11. Singleton VL, Orthofer R, LamuelaRaventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152–78
12. Aydin E. Evaluation of phenolic acid, total phenolic content, antioxidant capacity and in-vitro simulated bioaccessibility of healthy snack: Aromatized pumpkin chips. Emirates J Food and Agric. 2022.
13. Kelebek H, Sevindik O, Selli S. (2019). LC-DAD-ESI-MS/MS-based phenolic profiling of St John's Wort Teas and their antioxidant activity: Eliciting infusion induced changes. J Liquid Chroma & Related Technol 2019; 42:(1-2), 9-15.
14. Özen M, Özdemir N, Filiz BE, Budak NH, Kök-Taş T. Sour cherry (Prunus cerasus L.) vinegars produced from fresh fruit or juice concentrate: Bioactive compounds, volatile aroma compounds and antioxidant capacities. Food chem,2020; 309, 125664.

15. Özdemir N, Pashazadeh H, Zannou O, Koca I. Phytochemical content, and antioxidant activity, and volatile compounds associated with the aromatic property, of the vinegar produced from rosehip fruit (Rosa canina L.). 2022; LWT, 154, 112716.
16. Karamaæ M, Kosiñska A, Pegg RB. Content of gallic acid in selected plant extracts. J Food Nutr Sci. 2006;15(1):55–8.
17. Pandey K, Rizv S. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5):270–8.
18. Aganga AA. Mosase KW. Tannins content, nutritive value and dry matter digestibility of Lonchocarpus capassa, Ziziphus mucronata, Sclerocarya birrea, Kirkia acuminata and Rhus lancea seeds. Ani Feed Scie and Tech.2003; 91: 107-113.
19. Pedneault K. Leonharts A. Gosselin A. Ramputh A. Arnason JT. Influence de la culture hydroponique de quelques plantes médicinales sur la croissance et la concentration en composés secondaires des organes végétaux.Texte de conférence. Canada. 2001. pp: 1-5.
20. Tajik N, Tajik M, Mack I, Enck P. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. Eur J Nutr. 2017;56(7):2215–44
21. Oussou KF, Guclu G, Sevindik O, Starowicz M, Kelebek H, Selli S. Comparative Elucidation of Aroma, Key Odorants, and Fatty Acid Profiles of Ivorian Shea Butter Prepared by Three Different Extraction Methods. Separations,2022; 9(9), 245.