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PHENOLIC COMPOUNDS, ORGANIC ACIDS, METHYLXANTHINES AND SOLUBLE SUGARS PROFILES IN COCOA PLACENTA

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cocoa by-product, fermentation, bioactive compounds, theobromine, caffeine, food ingredient

ABSTRACT

Obtaining market cocoa beans generated by-products considered as waste discarded in the plantations. Among these, there is the placenta on which the beans are attached. This study aimed to identify and quantify by HPLC some metabolites (phenolic compounds, organics acids,, methylxanthines and soluble sugars) in powders from unfermented and fermented cocoa placenta. Profile of phenolic compounds showed a number of 14 and 11, respectively for the unfermented and fermented extracts. These were essentially the major classes of phenolic compounds, namely, phenolic acids, flavonoids and tannins, with the addition of coumarin and hydroquinone. Catechin displayed the highest level of 0.6346 ± 0.0004 mg/kg in unfermented placenta whereas condensed tannins scored highest concentration of 0.0736 ± 0.0005 mg/kg in fermented placenta. Regarding organic acids, the HPLC-profile allowed the detection of fumaric, lactic, oxalic, citric, acetic and tartaric acids. Quantitatively, lactic and acetic acids were the major organic acids in both cocoa placenta with respective contents of 5.5179 ± 0.0001 and 1.2036 ± 0.0004 mg/kg in the unfermented placenta; 5.6519 ± 0.0004 and 1.3830 ± 0.0003 mg/kg in the fermented placenta. HPLC analysis of methylxanthines, showed the presence of theobromine and caffeine in unfermented and fermented placenta. Theobromine was the predominant methylxanthine with 0.0975 ± 0.0013 and 0.0464 ± 0.0004 mg/kg for unfermented and fermented placenta, respectively. Respect to soluble sugars, the HPLC analysis showed the presence of glucose, fructose and sucrose in both cocoa placenta. In fermented placenta, fructose exhibited the highest due to its low fermentability, well below that of glucose. The presence of these metabolites found in cocoa beans could suggest the use of cocoa placenta powders as ingredients in the development of new cocoa-based food derivatives.

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ПРОФИЛИ ФЕНОЛЬНЫХ СОЕДИНЕНИЙ, ОРГАНИЧЕСКИХ КИСЛОТ, МЕТИЛКСАНТИНОВ И РАСТВОРИМЫХ САХАРОВ В ПЛАЦЕНТЕ КАКАО

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КЛЮЧЕВЫЕ СЛОВА:
плацента какао, ферментация, фенольные соединения, органическая кислота, метилксантин, растворимый сахар

АННОТАЦИЯ

Рассматривается вопрос получения рыночных какао-бобов из побочных продуктов, считающихся отходами, которые подлежат утилизации на плантациях. В число таких отходов включена растительная плацента, к которой крепятся бобы какао. Целью данного исследования было выявление и количественное определение с помощью ВЭЖХ анализа некоторых метаболитов (фенольных соединений, органических кислот, метилксантинов и растворимых сахаров) в порошках из неферментированной и ферментированной плаценты какао. Профиль фенольных соединений показал их наличие в количестве 14 и 11 в неферментированных и ферментированных экстрактах, соответственно. Представлены основные классы фенольных соединений, а именно, фенольные кислоты, флавоноиды и танины, с добавлением кумарина и гидрохинона. Катехин показал самый высокий уровень содержания — 0,6346 ± 0,0004 мг/кг — в неферментированной плаценте, тогда как в ферментированной плаценте самую высокую концентрацию показали конденсированные танины — 0,0736 ± 0,0005 мг/кг. Что касается органических кислот, анализ профиля посредством ВЭЖХ позволил обнаружить фумаровую, молочную, щавелевую, лимонную, уксусную и винную кислоты. Количественно молочная и уксусная кислоты были основными органическими кислотами в обоих образцах плаценты какао с соответствующим содержанием 5,5179 ± 0,0001 и 1,2036 ± 0,0004 мг/кг в неферментированной плаценте; и 5,6519 ± 0,0004 и 1,3830 ± 0,0003 мг/кг в ферментированной плаценте. Анализ содержания метилксантинов посредством ВЭЖХ показал наличие теобромина и кофеина в неферментированной и ферментированной плаценте. Теобромин был преобладающим метилксантином с долей содержания 0,0975 ± 0,0013 в неферментированной, и 0,0464 ± 0,0004 мг/кг в ферментированной плаценте, соответственно. Что касается растворимых сахаров, ВЭЖХ анализ показал наличие глюкозы, фруктозы и сахарозы в обеих плацентах какао. В ферментированной плаценте фруктоза показала наивысшую концентрацию по причине своей слабой ферментируемости, значительно более низкой, нежели чем у глюкозы. Наличие этих метаболитов, обнаруженных в какао-бобах, дает основания предполагать использование порошка из плаценты какао в качестве ингредиентов при разработке новых производных продуктов питания на основе какао.

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БЛАГОДАРНОСТИ: Авторы выражают благодарность местному населению Центрально-Западного региона Кот-д'Ивуара, и техническим специалистам Университета Нангуи Аброгуа за сбор образцов и оказание технической поддержки при проведении анализа методом высокоэффективной жидкостной хроматографии соответственно.

1. Introduction

The cocoa tree (*Theobroma cacao* L.) is the only plant whose commercial use of its beans from fruits, called, pods allows the production of chocolate [1]. It belongs to the Malvaceae family and is native to South and Central America [2] where it was cultivated by the Mayans and the Aztecs who called it "the food of God" [1]. Currently, cocoa is of inestimable economic importance in some developing countries because of its high contribution to the Gross Domestic Product (GDP) [3]. Côte d'Ivoire, Ghana and Indonesia are, respectively, the first, second and third producer. These three countries supply approximately 67% of the production of market cocoa beans [2]. Obtaining market cocoa beans generates enormous quantities of by-products considered as wastes, generally utilized in plantations, thus contributing to environmental pollution [4]. This waste is made up of three fractions, namely the pod husk, the placenta and the mucilage juice. Manufacturing of market cocoa beans into chocolate and derived products generates wastes called bean shells which constitute 12–20% of the cocoa seed [5]. Today, it well known that the valorisation of agricultural by-products wastes has become essential due to their significant volume generated by industrial processing and their richness in beneficial natural components [6,7]. Mendoza-Meneses et al. [8] indicated that the cocoa industrial wastes represent a source of usable biomass for the development of new products such as food, livestock feed, cosmetics, and chemical products, and they can even be used for the generation of biofuels. Thus, cocoa by-products were widely. Regarding to pod husk and bean shell, several reports showed that these cocoa by-products represent valuable bioactive compounds such as pectin, dietary fibers and phenolic compounds [5,9,10]. As for mucilage juice, studies conducted by Anvoh et al. [11] allowed it transformation into marmalade comparable to commercial products such as apricot marmalade. With respect to cocoa placenta which represents 2.58 ± 0.22% of the total fruit weight [12], investigations carried out on samples obtained from Côte d'Ivoire indicated that it could be good sources of macronutrients, minerals and antioxidant components [13]. In Côte d'Ivoire, the placenta is the waste resulting from extracting the market beans, which placenta is discarded on cocoa tree plantations. This waste can be obtained after the removal of the beans which subsequently undergo the fermentation stage. But also, the placenta can be fermented concomitantly with the beans which will subsequently be removed. Furthermore, it is well established that soluble fermentable sugars, phenolic compounds, alkaloids, notably methylxantines and organic acids, are strongly involved in obtaining tastes; aroma flavours, antioxidant and neurostimulator properties of products derived from cocoa beans, such as chocolate and cocoa powder [14–16].

This study aims to identify and quantify by chromatographic analysis using high-performance liquid chromatography coupled with a UV-visible Diode Array Detector or Refractive Index Detector (RID), the different phenolic compounds, organic acids, methylxanthines (theobromine, caffeine and theophylline) and soluble sugars (glucose, fructose and sucrose) contained in powders of this by-product in the fermented or unfermented condition. This can guide the food industry towards a possible using of these powders in the development of new cocoa-based products.

2. Materials and methods

2.1. Materials

Cocoa material. The cocoa material subject to this study was the placenta from ripe pods harvested from the Forastero variety in a plantation in the Gôh region, in the center-west of Côte d'Ivoire.

Chemicals. Standards of organic acids were provided by Sigma-Aldrich (Steinheim, Germany). On the other hand, the standards of phenolic compounds and the acetonitrile HPLC grade, standards of caffeine (99% purity), theobromine (98% purity) and theophylline (99% purity) were obtained from Merck (Darmstadt, Germany). Analytical grade powder of D-(−)-Fructose (⩾ 99%), D-(+)-Glucose (⩾ 99.5%) and sucrose (⩾ 99.5%) standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Methods

2.2.1. Cocoa placenta powder preparation

The cocoa placenta powders were obtained according to the method previously described by Goudé et al. [13]. The first batch containing all the placentas fermented concomitantly with the beans for 3 days, according to traditional fermentation methods, underwent separation with the beans. For fermentation, approximately 50 kg of placenta with their beans from the previously broken cocoa pods were poured onto plantain leaves laid on the ground. Then, the pile was covered with banana leaves and fermentation lasted three days. This placenta batch was dried in oven UFB400 (Memmert, Germany) at temperature 45 °C for 3 to 5 days. Subsequently, the dried placenta batch was ground and sieved (sieve of mesh 250 µm) to obtain the fermented cocoa placenta powder. The second batch containing all the unfermented placentas underwent the same treatments to obtain the unfermented placenta powder.

2.2.2. Phenolic compounds extract preparation

A volume of 50 mL of 80% (V/V) methanol was added to 10 g of each of the two previously prepared placenta powders. Then by stirring, the phenolic compounds were extracted at 35 °C for 24 hours, and then filtered through Whatman N°4 paper. This operation was repeated twice on the residue obtained. The three methanolic extracts thus obtained were combined to undergo evaporation at 35 °C using a rotary evaporator (HEILDOLPH Laborata 4003 Control rotary evaporator, Schwabach, Germany) down to 25 mL These 25 mL of each of the unfermented and fermented placenta extracts were diluted in 50 mL of distilled water and stored at −18 °C for the HPLC analysis.

2.2.3. Organic acid extract preparation

The organic acids of cocoa placenta powders were obtained according to Hasib et al. [17]. Briefly, 2 g of cocoa placenta powder was introduced into a centrifuge tube containing 20 mL of distilled water. This mixture was centrifuged (Biocen 22 R, Ortoalresa, Spain) at 4000 rpm for 30 min. The supernatant obtained was filtered through Whatman N°. 4 paper and then through a 0.45 µm Millipore filter (Millipore; Sartorius AG, Goettingen, Germany) and stored at −18 °C for further use in the HPLC analysis.

2.2.4. Methylxantine extract preparation

A mass of 2 g of placenta powder was mixed with 10 mL of 10% (V/V) methanol. This mixture was sonicated in an ultrasonic bath ULTR-2L0-001 (Labbox, France) during 30 min acceding to Júnior et al. [18]. Subsequently, the blend was centrifuged (Biocen 22 R, Ortoalresa, Spain) for 10 min at 3000 rpm, filtered through an Agilent 0.2µm syringe filter, and the supernatant transferred to a vial and maintained at a temperature of −18 °C.

2.2.5. Soluble sugar extracts preparation

Soluble sugars from cocoa placenta powders (fermented or unfermented) were extracted using the method of Rolland [19]. Briefly, a lump of 20 mg of cocoa placenta powder was added to 200 µL of 80% (V/V) ethanol in a test tube. The mixture was vigorously shaken for 10 min, followed by centrifugation (Biocen 22 R, Ortoalresa, Spain) at 13,000 rpm at room temperature during 3 min. The supernatant obtained which contains glucose, fructose and sucrose was kept. This operation was repeated 3 times. The extracts were then evaporated in an evaporator (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany) and diluted in 600 µL of ultra-pure water.

2.3. HPLC procedures

2.3.1. Phenolic compound analysis

The 50 mL of phenolic extracts prepared — were added in 100 ml of distilled water and 20 µl of each extract was injected in an HPLC unit (HPLC (Shimadzu Corporation, Japan) endowed with a binary pump (LC-6A) coupled to a UV–VIS detector (SPD-6A). Phenolic separation was performed on an Agilent Zorbax SB-C18 column (5µm particle size, 4.6 × 150 m, CA, USA) at 38 °C. The mobile phase was formic acid/distilled water (95/5; v/v) (eluent A) and acetonitrile/distilled water (98/2; v/v). The operation carried out with a flow rate of 1 mL/min lasted 25 min. Standard solution injection under the same conditions allowed to identify the different phenolic compounds by comparing retention times and UV-visible spectra. Peak areas of each phenolic and external calibration with standards were used for quantification.

2.3.2. Organic acid analysis

Organic acid analysis consisted of injecting 20 µL of organic acid extract from placenta powder into an analytical HPLC unit (Shimadzu Corporation, Japan) connected to a heating column device maintained at 35 °C via an oven (Meta Therm TM, Interchrom, France). The separation column used was an ICSep ICE ORH-801 ion exclusion column (40 cm × 5 µm, Interchrom, France). The system also included a pump (Shimadzu LC-6A Liquid Chromatograph) to circulate the mobile phase, a UV detector (Shimadzu SPD-6A UV Spectrophotometric Detector) and an

integrator (Shimadzu Chromatopac CR6A). Organic acids were detected at 210 nm and identified by comparing the retention times and spectral data obtained from standards under the same conditions. Quantification to obtain the different organic acid contents was carried out by comparing the peak areas with those of the external standards.

The main methylxanthines (theobromine, caffeine and theophylline) found in cocoa beans were explored chromatographically in placenta powders using an analytical HPLC unit (Shimadzu Corporation, Japan). The device also included a binary pump (LC-6A) associated with a UV–Vis detector (SPD-6A). Each of these methylxanthines was isolated using a reversed phase Hypersil™ ODS C18 Column with 10µm as particle size (Oslo, Norway). Analysis were carried out with isocratic elution at room temperature, injecting 10 µL of solution of cocoa placenta powder, using methanol — water — acetic acid (80:19:1) (v/v) as the mobile phase at a flow rate of 0.7 ml/min. UV-vis spectrophotometric detection was done at 273 nm. Theobromine solutions of concentrations 0.05; 0.100 and 0.250 mg/mL, caffeine and theophylline of concentrations 0.016, 0.040 and 0.250 mg/mL were prepared and injected under the same chromatographic conditions. Retention times and spectral data of standards collected under identical conditions were used for the identification of methylxanthines in cocoa placenta powders. Concentration of each compound was obtained through its peak area projected to the mean of its external standard.

2.3.3. Soluble sugar analysis

The analyses of soluble sugars (glucose, fructose and sucrose) of the cocoa placenta powders were carried out according to methods described by Jayawardhane et al. [20] and Md Saad et al. [21] with few modifications. The liquid chromatography performed involved the same device used for methylxanthines, but this time connected to refractive index detector (RID). Glucose, fructose and sucrose were separated with a reversed phase column ZORBAX (Agilent) NH_2 column, 5µm (4.5×250mm) in isocratic mode at temperature of 33 °C. The mobile phase used was a mixture of acetonitrile and water (75:25) at flow rate of 1 mL/min. A standard solution of each soluble sugar was previously prepared by dissolving 10 mg (glucose, fructose and sucrose) in the acetonitrile/water mixture (2/1), and then filtered through 0.45µm nylon filters. Concentrations of 0.3:1.5 and 5.00 mg/mL of each standard were prepared by diluting each stock solution. The separation of the different soluble sugars was obtained by injecting 10 µL of the standards and samples of cocoa placenta powder were injected into an NH_2 column according to the following procedure. First, the different standard solutions at 5 mg/mL were injected separately to estimate their retention time. Then, the mixture at the same concentration and equal volume was injected for comparative analysis. Finally, each of the cocoa placenta powder samples was injected to detect and identify soluble sugars by comparison of retention times. The quantification of the concentration of each sugar expressed in concentration (mg/kg) was achieved by relating the area of the peak to that of its standard at a given concentration. Subsequently, the concentration of glucose, fructose and sucrose in the placenta powder was obtained by calculating the average of the values found previously.

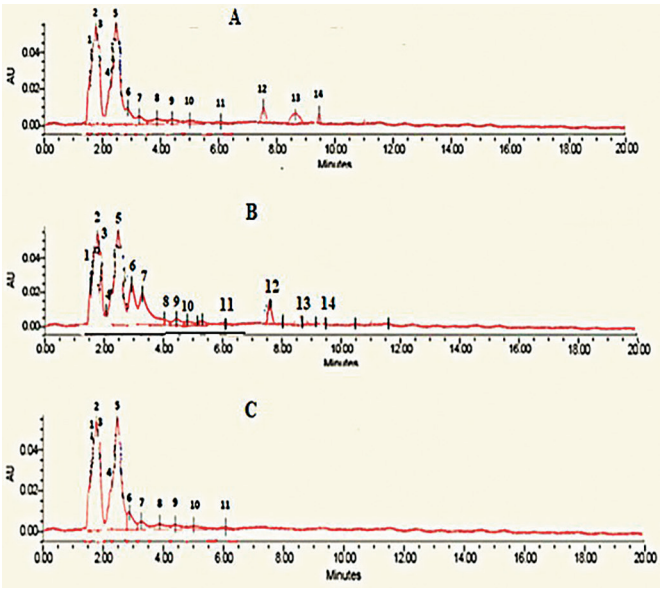

Figure 1. **Phenolic compounds HPLC-profiles of of methanolic extracts from cocoa placenta powders; A: standards of phenolic compound, B: unfermented placenta, C: fermented placenta; detection at 280 nm: (1) tannin-H2O; (2) tannin-ol; (3) gallic acid; (4) cinnamic acid; (5) catechin; (6) flavone; (7) caffeic acid; (8) pyrocatechol; (9) coumarine; (10) quercetine; (11) hydroquinone; (12) Figure ferulic acid; (13) veratric acid; (14) rutin**

Рисунок 1. **ВЭЖХ-профили фенольных соединений метанольных экстрактов из порошков плаценты какао; A: стандарты фенольных соединений, B: неферментированная плацента, C: ферментированная плацента; порог обнаружения — при 280 нм: (1) танин-H2O; (2) танин-ол; (3) галловая кислота; (4) коричная кислота; (5) катехин; (6) флавон; (7) кофейная кислота; (8) пирокатехин; (9) кумарин; (10) кверцетин (11) гидрохинон; (12) феруловая кислота; (13) вератровая кислота; (14) рутин**

2.4. Statistical analysis

All data were derived from the average of three analyses. Values obtained were as means ± standard deviation (SD). Analysis of variance (ANOVA) and Tukey's test were used to test for differences between means by Statistica 7.1 software. Significant differences were considered at the 5% level ($p \leq 0.05$).

3. Results and discussion

3.1. Phenolic compounds in cocoa placenta powders

HPLC analysis allowed to separate and identify, on the one hand, 14 phenolic compounds in the unfermented placenta powder and, on the other hand, 11 in the fermented one (Figure1). These phenolic compounds detected in unfermented placenta powder belonged to several classes, namely tannins (tannins ol, i. e. condensed tannins; tannins H_2O, i. e. hydrolysable tannins), phenolic acids (gallic acid, cinnamic acid, caffeic, ferulic acid, veratric acid), flavonoids (catechin, flavone, pyrocatechol quercetine, rutin), coumarin and hydroquinone. On the other hand, the fermentation process caused the disappearance of ferulic acid, veratric acid and rutin in fermented placenta powder. It is well established that cocoa beans and derived products such as chocolate have always been good sources of phenolic compounds, particularly phenolic acids, flavonoids and proanthocyanidines which are in fact condensed tannins also called catechic tannins [14,22–24]. The same was true for some by-products such as pod husk [25,26] and cocoa beans shells [5,27,28]. The disappearance of some phenolic compounds noted during fermentation in this study seems to be a common occurrence in cocoa. Indeed, previous reports have indicated similar results during fermentation of cocoa beans [23,29,30]. In terms of contents, Table 1 showed that catechin displayed the highest value of 0.6346 ± 0.0004 g/100 g in extract of unfermented cocoa placenta powder, followed by hydroquinone (0.1339 ± 0.0003 g/100 g) and condensed tannins (0.1039 ± 0.0004 mg/kg). On the other hand, in the fermented powder extract, it was the condensed tannins that scored the highest concentration (0.0736 ± 0.0005 mg/kg), followed by pyrocatechol (0.0564 ± 0.0005 mg/kg) and hydrolysable tannins (0.0328 ± 0.0003 mg/kg). Upon examination of these contents of the

Table 1. **Individual phenolic compounds contents (mg/kg) in unfermented and fermented cocoa placenta powders**

Таблица 1. **Содержание отдельных фенольных соединений (мг/кг) в неферментированных и ферментированных порошках плаценты какао**

Phenolic compound	Peak	RT (min)	Cocoa placenta powder, mg/kg	
			Unfermented	Fermented
Tannin-H_2O	1	1.566	0.0354 ± 0.0005^a	0.0328 ± 0.0003^b
Tannin-ol	2	1.779	0.1039 ± 0,0004^a	0.0736 ± 0,0005^b
Gallic acid	3	1.820	0.0147 ± 0,0004^a	0.0136 ± 0.0004^a
Cinnamic acid	4	2.284	0.0040 ± 0.0003^b	0.0029 ± 0.0004^b
Catechin	5	2.474	0.6346 ± 0.0004^a	0.1360 ± 0.0004^b
Hydroquinone	6	2.862	0.1339 ± 0.0003^a	0.0025 ± 0.0004^b
Caffeic acid	7	3.247	0.0640 ± 0,0003^a	0.0069 ± 0.0003^b
Pyrocatechol	8	3.869	0,0185 ± 0.0003^a	0.0564 ± 0.0005^b
Coumarine	9	4.409	0.0050 ± 0.0001^a	0.0170 ± 0.0003^b
Quercetine	10	5.012	0.0021 ± 0.0001^a	0.0021 ± 0.0001^a
Flavone	11	6.073	0.0048 ± 0.0002^a	0.0553 ± 0.0005^b
Rutin	12	7.488	0.0031 ± 0.0002^a	nd
Ferulic acid	13	8.624	0.0816 ± 0.0004^a	nd
Veratric acid	14	9.602	0.0019 ± 0.0003^a	nd

RT: Retention Time, nd: non-detect.
Each value is from an average of three trials. Values are the mean ± standard deviation. In each row, mean values not bearing the same letter are significantly different at $p \leq 0.05$ according to Tukey's test).

different phenolic compounds in the powder of unfermented and fermented placenta, the trend which emerged overall was that a decrease in them was observed during fermentation. According to some reports, this loss of phenolic compounds could be attributed to their leaching with the fermentation exudate and also to their oxidation by polyphenol oxidases as clearly shown by the brown colour of the fermented placenta [31,32]. Obviously, this could have a negative impact on the antioxidant capacity of the fermented cocoa placenta powder which will decrease considerably compared to that which is not fermented as reported by Fang et al. [33] and Melo et al. [34] for cocoa beans.

3.2. Organic acids in cocoa placenta powders

The unfermented and fermented cocoa placenta powder extracts exhibited an organic acid profile consisting of six identified organic acids which were fumaric acid, lactic acid, oxalic acid, citric acid, acetic acid and tartaric acid (Figure 2). In general, these organic acids have previously been detected in cocoa beans [35–37] and in some by-products [11,38]. High levels of acetic acid and lactic acid are reported to be responsible for the acid flavor of cocoa products from certain regions [36].

In terms of quantity, as shown in Table 2, lactic and acetic acids were the major organic acids in both cocoa placenta powders with respective contents of 5.5179 ± 0.0001 and 1.2036 ± 0.0004 mg/kg in the unfermented powder; 5.6519 ± 0.0004 and 1.3830 ± 0.0003 mg/kg in the fermented powder. These two acids originated from the fermentative activity of lactic acid bacteria on the one hand and acetic acid bacteria on the other hand [39–41]. Organic acids such as citric and oxalic acids, generally present in fermented cocoa beans [42], were also present in both powders with respective significant levels of 0.4388 ± 0.0005 and 0.2756 ± 0.0004 mg/kg in the unfermented powder; 0.4641 ± 0.0004 and — 0.2421 ± 0.0004 mg/kg in the fermented powder. Fumaric acid was detected in fermented powder with a relatively considerable content of 1.1055 ± 0.0004 mg/kg. Overall, contents of different organic acids in cocoa placenta were significantly higher in the fermented powder than in the unfermented one, with the exception of citric acid. In fact, organic acids are produced during the fermentation process by acetic acid bacteria, lactic acid bacteria and yeasts and this must require increasing the pH of the fermentation medium through the reduction of the citric acid content [43,44]. Some studies have even reported that raw unfermented cocoa bean did not contain lactic and acetic acids [45,46]. In the case of this present study, it would be very likely that the fermentation of the placenta began early inside the pod. Moreover, despite the appearance of organic acids such as lactic, acetic and oxalic acids throughout fermentation, the difference in various acids content between fermented and unfermented placenta, although statistically significant, was not sufficiently considerable. This could be attributed to the fact that these acids could be carried away into the fermentation exudate due to the low stiffness of the cocoa placenta. In fact, the fermentation exudate corresponded to the juice of the fermenting mucilage. The finding of Anvoh et al. [11] previously confirmed a presence of considerable quantity of organic acids in cocoa fermented juice.

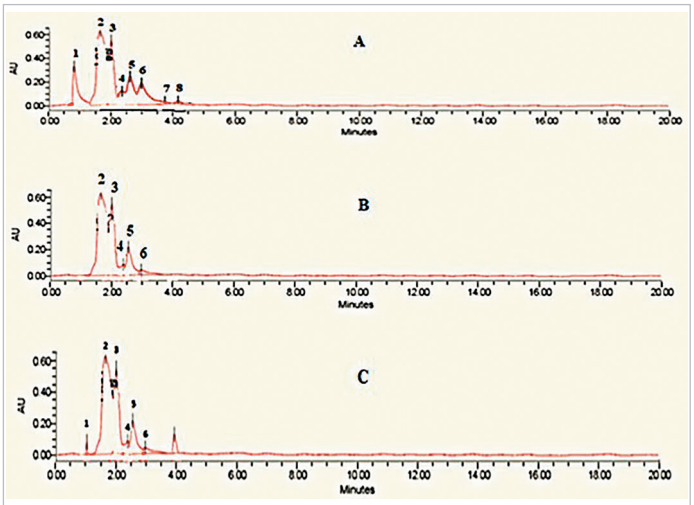

Figure 2. **Organic acid HPLC-profiles of extracts from cocoa placenta powders; A: standards of organic acid, B: unfermented placenta, C: fermented placenta; detection was made at 210 nm: the numbers (1), (2), (3), (4), (5), (6), (7) and (8) designate respectively fumaric, lactic, oxalic, citric, acetic, tartaric, succinic and tannic acids**

Рисунок 1. **Профили ВЭЖХ органических кислот экстрактов из порошков плаценты какао; А: стандарты органических кислот, В: неферментированная плацента, С: ферментированная плацента; порог обнаружения — 210 нм: цифры (1), (2), (3), (4), (5), (6), (7) и (8) обозначают соответственно фумаровую, молочную, щавелевую, лимонную, уксусную, винную, янтарную и таниновую (дубильную) кислоты**

Table 2. **Individual organic acid contents (mg/kg) in unfermented and fermented cocoa placenta powders**

Таблица 2. **Содержание отдельных органических кислот в порошке неферментированной и ферментированной плаценты**

Organic acid	Peak	RT (min)	Cocoa placenta powder, mg/kg	
			Unfermented	Fermented
Fumaric acid	1	1.401	nd	1.1055 ± 0.0004[a]
Lactic acid	2	1.671	5.5179 ± 0.0001[a]	5.6519 ± 0.0004[b]
Oxalic acid	3	1.990	0.4388 ± 0.0005[a]	0.4641 ± 0.0004[b]
Citric acid	4	2.404	0.2756 ± 0.0004[a]	0.2421 ± 0.0004[b]
Acetic acid	5	2.611	1.2036 ± 0.0004[a a]	1.3830 ± 0.0003[b]
Tartaric acid	6	3.102	0.0932 ± 0.0004[a]	0.0996 ± 0,0005[b]
Succinic acid	7	3,944	nd	Nd
Tannic acid	8	4,082	nd	Nd

RT: Retention Time, nd: non-detected.
Each value is from an average of three trials. Values are the mean ± standard deviation. In each row, mean values not bearing the same letter are significantly different at $p \leq 0.05$ according to Tukey's test.

3.3. Methylxanthines in cocoa placenta powders

Caffeine (1,3,7-trimethylxanthine, theobromine (3,7-dimethylxanthine) and theophylline (1,3- dimethylxantine) are alkaloids of methylxanthines class naturally present in cocoa bean [47,48]. The latter have very close chemical structures and have been the subject of numerous researches. They are well known for their stimulant effects on the central nervous and cardiovascular systems [47,49]. The existence of theobromine and caffeine has been highlighted in some by-products such as cocoa bean shell [3,50] and cocoa mucilage [3]. In the present study, theobromine, caffeine and theophylline were successfully analyzed in fermented and unfermented cocoa placenta powders. The results presented on the chromatographic profile (Figure 3) showed that the unfermented cocoa placenta presented peaks of theobromine, caffeine and theophylline. On the other hand, for the placenta fermented in cocoa powder, although exhibiting peaks of theobromine and caffeine, theophylline is completely absent.

Regarding concentrations of these different methylxanrhines presented in Table 3, unfermented cocoa placenta powder showed values of 0.0975 ± 0.0013; 0.0607 ± 0.0004 and 0.0193 ± 0.0002 mg/kg for theobromine, caffeine and theophylline, respectively. As for fermented cocoa placenta powder, respective concentrations of 0.0464 ± 0.0004 and 0.0295 ± 0.0004 mg/kg were recorded for theobromine and caffeine while theophylline was absent. These data from the analysis of methylxanthines showed overall that cocoa placenta contains significant levels of theobromine, caffeine and traces of theophylline like cocoa beans. In addition,

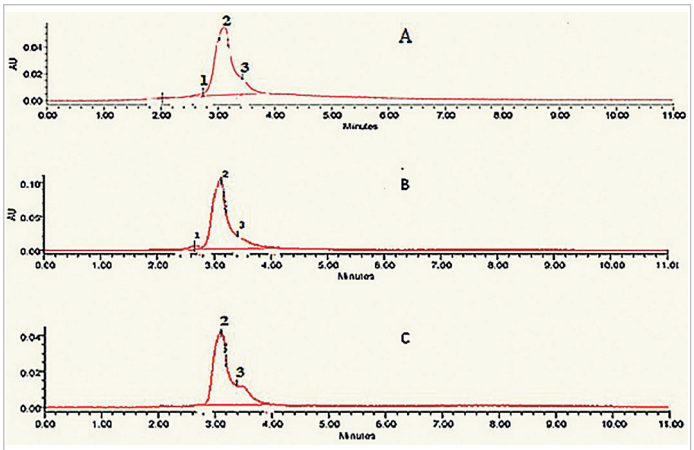

Figure 3. **Methylxanthine HPLC-profiles of extracts from cocoa placenta powders; A: standards of methyxanthine, B: unfermented placenta, C: fermented placenta; detection at 273 nm: (1) theophylline; (2) theobromine; (3) caffeine**

Рисунок 3. **ВЭЖХ профили метилксантина экстрактов из порошков плаценты какао; А: стандарты метиксантина, В: неферментированная плацента, С: ферментированная плацента; порог обнаружения при 273 нм: (1) теофиллин; (2) теобромин; (3) кофеин**

Table 3. **Methylxanthine contents (mg/kg) in unfermented and fermented cocoa placenta powders**

Таблица 3. **Содержание метилксантина (мг/кг) в порошках неферментированной и ферментированной плаценты какао**

Methylxanthine	Peak	RT (min)	Cocoa placenta powder, mg/kg	
			Unfermented	Fermented
Théophylline	1	2.659	0.0193 ± 0.0002	nd
Théobromine	2	3.116	0.0975 ± 0.0013[a]	0.0464 ± 0.0004[b]
Caffeine	3	3.413	0.0607 ± 0.0004[a]	0.0295 ± 0.0004[b]

RT: Retention Time, nd: non-detect.
Each value is from an average of three trials. Values are the mean ± standard deviation. In each row, mean values not bearing the same letter are significantly different at $p \leq 0.05$ according to Tukey's test.

fermentation induced a considerable drop in methylxanthine content as has been noted for cocoa beans in the literature [18,51–53]. This decrease in methylxanthine levels could be explained by the fact that during fermentation, the pulp, that is to say the mucilage attached to the bean and the placenta which contains theobromine and caffeine [51], is extracted with the aid from the increase in temperature and leached into the fermentation exudate as has been reported for the cocoa bean [54].

3.4. Soluble sugars in cocoa placenta powders

According to some authors, the profile of soluble sugars in cocoa raw products constitutes a quality index; these sugars are found to be one of the main precursors of flavor and taste for which the newly formed compounds from these sugars are responsible [55,56], all of which justifies the analysis of fructose, glucose and sucrose in this study. Figure 4 showed the chromatographic profiles of these soluble sugars in fermented and unfermented cocoa placenta powders. It was observed that these three soluble sugars were detected in both cocoa placenta powders. It is well

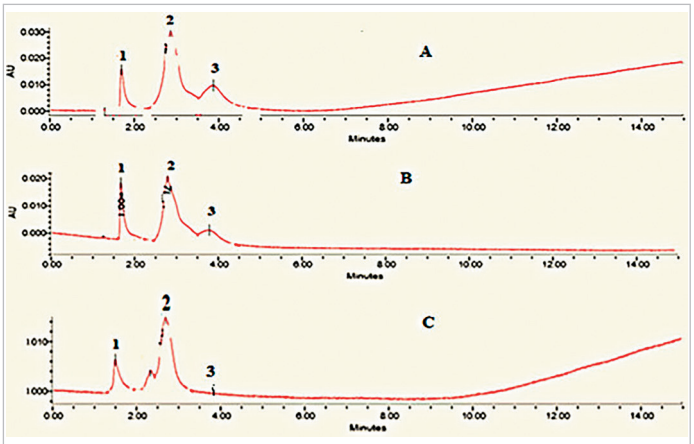

Figure 4. **Soluble sugars HPLC-profiles of extracts from cocoa placenta powders; A: standards of soluble sugars, B: unfermented placenta, C: fermented placenta; detection with Refractive Index Detector (RID: (1) Fructose; (2) Glucose (3) Sucrose**

Рисунок 4. **Растворимые сахара. ВЭЖХ профили экстрактов из порошков плаценты какао; A: стандарты растворимых сахаров, B: неферментированная плацента, C: ферментированная плацента; обнаружение с помощью рефрактометрического детектора (RID): (1) фруктоза; (2) глюкоза (3) сахароза**

Table 4. **Sucrose, glucose and sucrose contents (mg/kg) in sugar extracts from cocoa placenta powders**

Таблица 4. **Содержание сахарозы, глюкозы и сахарозы (мг/кг) в экстрактах сахара, извлеченных из порошков плаценты какао**

Soluble sugars	Peak	RT (min)	Sugar extract from cocoa placenta powder, mg/kg	
			Unfermented batch	Fermented batch
Fructose	1	1.709	0.2558 ± 0.0002[a]	0.2075 ± 0.0004[b]
Glucose	2	2.870	0.1681 ± 0.0002[a]	0.1299 ± 0.0004[b]
Sucrose	3	3.801	0.0145 ± 0.0003[a]	0.0076 ± 0.0006[b]

RT: Retention Time.
Each value is from an average of three trials. Values are the mean ± standard deviation. In each row, mean values not bearing the same letter are significantly different at $p \leq 0.05$ according to Tukey's test.

known that sucrose, glucose and fructose are the predominant sugars in fresh cocoa beans [57,58]. These soluble sugars were also detected in some cocoa by-products such as mucilage [59].

In terms of quantification, Table 4 shows concentration of each soluble analyzed sugar. Fructose exhibited the higher concentration in unfermented placenta powder estimated to 0.2558 ± 0.0002 mg/kg, followed by glucose with a value of 0.1681 ± 0.0002 mg/kg. As for sucrose, its concentration in unfermented placenta powder was the lowest with an estimated value of 0.0145 ± 0.0003 mg/kg. The presence of these three soluble sugars has already been reported in unfermented cocoa beans [60,61]. Sucrose, glucose and fructose are mainly found in the pulp which covers the beans and the placenta, and during fermentation these sugars are metabolized by yeasts [57]. Furthermore, previous reports have indicated that sucrose is the predominant soluble sugar in fresh pulp before the fermentation [61,62].

On the other hand, in the present study, sucrose had the lowest concentration in the unfermented cocoa placenta. This might be attributed to premature fermentation in the placenta. Regarding fermented cocoa placenta powder, it was overall observed that the concentrations of the different sugars decreased to reach values of 0.2075 ± 0.0004; 0.1299 ± 0.0004 and 0.0076 ± 0.0006 mg/kg at the end of fermentation for fructose, glucose and sucrose respectively. This observation is in agreement with several others during the fermentation of cocoa beans [57,61]. As reported earlier in this study, this could be justified by the fact that these sugars are fermented by microorganisms to produce various metabolites such as ethanol, organic acids and others [63]. In addition, during fermentation, sucrose was almost hydrolyzed to glucose and fructose by endogenous invertase [55,64]. Furthermore, the concentration of fructose is the highest in fermented cocoa placenta powder; this could be ascribed to the preferential metabolism of glucose during fermentation [65].

4. Conclusion

The results reported in this study clearly indicated that in terms of phenolic compounds, organic acids, methylxanthines and soluble sugars, the compositions of the fermented cocoa placenta powder were approximately similar to cocoa powder from the fermented beans. This could be a good quality indicator that could allow this by-product of obtaining market cocoa beans to be considered as an ingredient for the development of cocoa-based food derivatives instead of fermented cocoa beans powder. This would constitute an added value in the valorization of the by-products of cocoa processing, as the placenta is generally discarded in the cocoa plantations.

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E. J. P. Kouadio designed the research concept, provided the analysis tools, wrote the manuscript, and submitted it. **K. A. Goudé**, **K. H. Kouamé** and **O. J Gbotognon** were responsible for data collection and analysis.	**Э. Дж. П. Куадио** разработал концепцию исследования, предоставил инструменты анализа, написал рукопись и представил ее на рассмотрение. **К. А. Гуде**, **К. Х. Куаме** и **О. Дж. Гботоньон** провели экспериментальные исследования и анализ данных
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