BIOCHEMICAL CHARACTERIZATION, NUTRITIONAL AND ANTIOXIDANT POTENTIALS OF COCOA PLACENTA (*THEOBROMA CACAO* L.)

Goude, Kouadio Alfred[1]; **Adingra,** Kouassi Martial-Didier[2], **Gbotognon,** Oscar Jaures[1] and **Kouadio,** Eugène Jean Parfait[1]*

[1]Laboratoire de Biocatalyse et des Bioprocédés,
Université Nangui Abrogoua (Abidjan, Côte d'Ivoire), 02 BP 801 Abidjan 02, Côte d'Ivoire
[2]Laboratoire de Biochimie des Aliments et de Transformation des Produits Tropicaux,
Université Nangui Abrogoua (Abidjan, Côte d'Ivoire), 02 BP 801 Abidjan 02, Côte d'Ivoire
**E-mail*: nkouadiop@yahoo.fr

Abstract
Fruit by-products are generally discarded after use of the edible portion. This work aims to contribute to the valorization of the placenta (a by-product) of cocoa. The mature cocoa harvested from a village plantation in West-Central Côte d'Ivoire has been used as a plant material. The biochemical tests performed on the placenta (fresh and fermented) extract cocoa pods with the rules of hygiene have shown significant levels of fiber, ash, minerals and antioxidant compounds. In particular, the fermented placenta contains more fibers (42.66%), ash (10.37%) and phenolic compounds including total phenols (37.5 mg EAG/100 g DW), flavonoids (3.05 mg EQ / 100 g DW) and tannins (10.86 mg EAT / 100 g DW). These fermented and fresh placentas also contain macroelements (K, Mg and P) and mineral microelements (Fe, Zn). The placenta from cocoa could be a good source of food for animal and human. The determination of the antioxidant activities by inhibition of ABTS and DPPH radicals showed interesting data (fresh placenta: ABTS 12.73 µMTE / g DW, DPPH 9.46 ± 0.53 µMTE / g DW, fermented placenta: ABTS 13 77 µMET / g MS, DPPH 13.05 µMTE / g DW). The antioxidant activity increases after fermentation of the cocoa placenta. As a result, cocoa placenta could be an ingredient in livestock feed formulation and even for humans.

Keywords: Placenta, Cocoa, Biochemical composition, Antioxidant activity, Fermentation

Received: 24.06.2019 Reviewed: 24.09.2019 Accepted: 25.09.2019

INTRODUCTION

Projections made in 2011 by the United Nations (UN) give for 2050 an increase of the world population of 36 % (Hamdouche, 2015). The growth of the world's population is leading to an increase in food needs (FAO, 2017). The satisfaction of the demand for agricultural production therefore passes either by the increase of the agricultural surfaces, or by the increase of the yields (Muzigwa *et al.*, 1995, Ngambi, 2015). Thus, the world consumption of chocolate being strong and constant, cocoa cultivation cannot be outdone by this need for intensification of production.

According to the International Cocoa Organization (ICCO), global cocoa production in 2017 was estimated at 4.7 million tonnes (ICCO, 2017). This production is made by farms of which 90% are less than 5 hectares. Cocoa production supports more than 5 million families (Hamdouche, 2015).

However, it is important to specify that fresh beans account for only 20% of pod mass (Kokou & Ngo-Samnick, 2014), while cocoa waste (empty cacao pods, juice from mucilage and cocoa placenta) account for 80 % of the mass of the pod. Most of the time, these wastes are released into the wild and rot in fields or scavenging sites. The accumulation of these residues pollutes the environment (Manga, 2013).

Recently, attempts to use these vegetable wastes have been made with a view to serving as food supplements or substitutes for animal feed or even for humans (Erhart & Poulain, 2014). Thus, the current valuation of by-products such as empty pods is used to make fertilizers, feed for animals (pork, poultry and fish) and as a component of recipes for humans (Pitcholo 1990, Muzigwa *et al.*, 1995; Iga-Iga, 2008; CIRAD, 2015; Oqualim, 2016 and Bamba *et al.*, 2017). As for the juice from the mucilage surrounding fresh cocoa beans, it is

used to make cocoa wine, cocoa liquor (Martens, 2011; CNRA, 2012). On the other hand, the cocoa placenta is little valued and rejected during the treatment of cocoa pods (Quimbita *et al.*, 2013). Like empty pods and mucilage juice, can the placenta, which is also a by-product of cocoa, not be useful to humans or animals? Can the cocoa placenta not be subject to a valuation policy with a view to solving food security issues? This byproduct may have nutrients useful for the dietary formulation of livestock feed or even human.

Thus, the aim of this study is to contribute to the valorization of the cocoa placenta in order to contribute to food security.

Specifically, it will to determine the biochemical and nutritional composition of the cocoa placenta, then evaluate the mineral composition and finally to determine the phytochemical composition and antioxidant activity.

MATERIALS AND METHODS

Plant material

The plant material comes from cocoa pods of the variety Forastero ripe freshly harvested in cocoa farms of Central West of Côte d'Ivoire during 2018 big season. From these pods is extracted the cocoa placenta which constitutes the plant material of this work.

Harvest

The harvest of cocoa pods was done by hand and with a perch. Harvested pods were packed in jute bags and transported from the picking place to Biocatalysis and Process Laboratory of Nangui Abrogoua University for analysis. The harvest was carried out in the month of September 2018. Three batches of samples of cocoa pods were necessary to carry out this work. The cocoa fruit was cut at the Biocatalysis and Process Laboratory and not in the harvest fields to prevent the beans from fermenting during the transport of the plant material.

Treatment of cocoa pods

Pod breaking

Each batch of cocoa pods was sorted and cleared of the damaged fruit.

The breaking was made with pieces of wood and pestles in the laboratory. The beans were removed from the pods with the placenta and all the mucilage surrounding them. They were stored in clean basins. Then, the placenta was removed from the bean mass under standard hygienic conditions fresh or after fermentation.

Two batches of bean and cocoa placenta samples were made according to these objectives:

- batch (A) which made it possible to carry out analyzes on the fermented cocoa placenta,
- batch (B) which has not been fermented but analyzed, fresh.

This batch (B) of cocoa beans and unfermented fresh placenta was subjected to the following operations. The placenta was separated from the cocoa beans. This fresh placenta was weighed and dried in an oven at 105 °C for 3 days. Batch (A) was fermented according to a method and a device described below.

Fermentation

Lot (A) of cocoa beans and placenta were fermented according to a traditional method (Manga, 2013; Hamdouche, 2015) whose device was created and installed in the laboratory. Banana leaves were used to cover the device. In addition, these banana leaves were also used to cover the beans and cocoa placenta during fermentation.

This fermentation was carried out for three days. After fermentation, the placenta was separated from the cocoa beans. The fermented placenta was dried in an oven at 105°C for 3 days.

Preparation of cocoa placenta powders

Oven dried cocoa placenta batches were removed from the oven.

Batch (A) consists of the fermented placenta and batch (B) of the unfermented placenta and dried in the fresh state. Then, the different batches of cocoa placenta were crushed into a fine powder using a grinder type Stand Blender Model BL 1008A-CB NASCO.

The ground materials obtained were sieved through a sieve of mesh 250 microns.

The two samples of placenta powder obtained were packaged in labeled plastic bottles. These flasks were pre-dried in an oven at 45°C for 15

minutes and sealed. These were stored in a desiccator at 25°C until their next use (AOAC 1995).

Determination of physicochemical parameters

Moisture was determined by drying in an oven at 105ºC during 24 h to constant weight (AOAC, 2000). pH was determined using a pH meter (Consort P107, Belgique). Total sugar and reducing sugar were determined by Dubois *et al.* (1956) and Bernfeld (1955), respectively. Total ash was determined by incinerating in a furnace at 550ºC (AOAC, 2000). Crude fat was determined by continuous extraction in a Soxhlet apparatus (Soxtec sostem HT 1043) for 8 h using hexane as solvent (AOAC, 2000). Crude protein was calculated from nitrogen (N x 6.25) obtained using the Kjeldahl method by AOAC (2000). The crude fibre contents were determined according to standard method (AOAC, 2000).

Minerals Analysis

The minerals contents were determined on aliquots of the ash of "placenta cocoa" using the Variable Pressure Scanning Electron Microscope (SEM) of the C.A.R.D. (MEB FEG Supra 40 VP Zeiss), equipped with an X-ray detector (OXFORD Instruments) connected to an EDS microanalyzer platform (Inca Dry Cool, without liquid nitrogen).

Calculation of the different molar ratios such as [phytate] / [calcium], [phytate] / [iron] and [oxalate] / [calcium] were used in the prediction of calcium and iron bioavailability (APHA, 1995).

Phytochemical Composition

Extraction of phenolic compounds

Extraction of phenolic compounds was determined employing Singleton *et al*. (1999) method. Ten (10) grams of each sample of the different batches of cocoa placenta were incubated at 25°C for 24 hours in 50 mL of 80 % (v/v) methanol. The mixtures obtained after incubation were centrifuged separately at 4000 rpm for 5 min with an ORTO ALRESA refrigerated centrifuge. The supernatants are recovered and the residues of each sample are extracted twice in succession under the same conditions. The different methanolic extracts are evaporated at 35°C until 50 ml of solution are obtained, using an evaporator (rotary evaporator HEILDOLPH Laborata 4003 Control, Schwabach, Germany). The different solutions were then transferred to new tubes and kept in the freezer at -20°C in the laboratory until use.

Determination of total phenolic compounds

Contents of total phenolic compounds were estimated according Folin-Ciocalteu method (Singleton *et al*., 1999). A volume of 1 ml of methanolic extract of each sample was added to 1 ml of Folin-Ciocalteu's solution in a test tube. After 3 minutes, 1 ml of 20% sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The mixture was allowed to stand at room temperature in a dark environment for 30 min. Absorbance was measured against the blank reagent at 725 nm. Gallic acid was used for the calibration curve with a concentration range of 50-1000 µg/ml. Results were expressed as mg gallic acid equivalent (GAE)/100g DW (Dry Weight).

Determination of flavonoids compounds

Total flavonoids content was determined according method used by Meda *et al.* (2005), but slightly modified. A volume of 0.5 ml of methanolic extract of sample was diluted in 0.5 ml of distilled water. Then, 0.5 ml of aluminium chloride 10% (P/V) and the same volume of sodium acetate 1M were added. Finally, 2 ml of distilled water was added and absorption reading at 415 nm was carried out after 30 min against a blank sample consisting of a 4 ml methanolic extract without aluminum chloride. Quercetin was used for the calibration curve with a concentration range of 0-100 µg/ml. Results were expressed as mg of quercetin equivalent (QE)/100g DW.

Determination of tannins compounds

Tannins content was determined using the method described by Bainbridge *et al*. (1996). A volume of 1 ml of each methanolic extract was collected and mixed with 5 ml of reaction solution [vanillin 0.1mg/ml in sulphuric acid 70% (V/V)]. The mixture was allowed to stand at room temperature in a dark environment for 20 min. The absorbance was measured at 500 nm against a blank (without extract). Tannic

acid was used for the calibration curve with a concentration range of 0-100 µg/ml. The results were expressed as mg of tannic acid equivalents (TAE)/100 g DW.

Determination of antioxidant activity

Antioxidant activity by inhibition of ABTS

Spectrophotometric analysis of the ability of methanolic cocoa placenta extracts to trap ABTS radicals was determined according to the method of Re *et al.* (1999). The preparation of the ABTS solution was carried out by dissolving 10 mg of ABTS in 2.6 ml of distilled water. Then, 1.7212 mg of potassium persulfate was added and the mixture was kept in the dark at room temperature for up to 12 hours. The mixture was then diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. In 96-well plates, 50 µl of ethanolic solution was added to 200 µl of freshly prepared ABTS + solution. The same procedure was carried out for quercetin used as a positive control. The mixture made in the 96-well plates was then protected from light in the dark at room temperature for 15 minutes and the concentration read at 734 nm in a spectrophotometer against a standard curve with 5,7,8-tetramethyl-2-carboxylic acid 6-hydroxy-2 (Trolox, Sigma-Aldrich). The evolution of the antioxidant activity of the extracts is compared with respect to Trolox. The concentration of the compounds having a reducing effect on the radical cation ABTS + (antioxidant) is expressed in micromoles equivalent Trolox per gram of dry matter (µMET / g DW) using the following formula: $C = (c_x D) / Ci$.

With: C, the concentration of antioxidant compounds in µMET / g; cx, the concentration of the sample read; D, the dilution factor and Ci, concentration of the stock solution.

Antioxidant activity by DPPH radical scavenging

The DPPH scavenging activity was determined using the method described by Shimada *et al.* (1992). Each sample of methanolic extract (2.5 ml) was mixed with 1 ml of a 3 mM DPPH methanol solution. After 30 min incubation at room temperature in the dark, the absorbance of the mixture was determined at 517 nm against a blank containing methanol without DPPH radical. A lower absorbance indicates a higher scavenging activity.

The evolution of the antioxidant activity of the extracts is compared with 5,7,8-tetramethyl-2-carboxylic acid 6-hydroxy-2 (Trolox) and this by drawing a calibration curve. 2.5 ml of Trolox solutions with a concentration of 0.01 to 0.1 g / l are prepared. Each solution was pipetted into test tubes followed by the addition of 1 mL of DPPH solution. The readings of the optical density at 517 nm, solutions thus prepared made it possible to trace the Trolox calibration curve. The concentration of the compounds having an inhibiting (antioxidant) effect on the DPPH is expressed in micromoles Trolox equivalent per gram of dry matter (µMTE / g DW).

Determination of antinutritional factors

Determination of total phytates and oxalates

Phytates contents were determined using the Latta & Eskin (1980) method. While the oxalates contents was determined using the method of Day & Underwood (1986) using potassium permanganate.

Statistical Analysis

All chemical analyses and assays were performed in triplicate, unless otherwise indicated. Results were expressed as mean values□± standard deviation (SD). Analysis of variance (ANOVA) followed by Duncan's test. The significance level is $\alpha = 0.05$. Statistical differences with a probability value less than 0.05 ($P < 0.05$) are considered significant.

RESULTS AND DISCUSSION

Cocoa is mainly produced for its bean for industrial processing into chocolate. However, more and more research is being conducted on cocoa waste for recovery and use in animal or human nutrition. In this perspective, the present work has made it possible to characterize the biochemical composition of the cocoa placenta. The results of physicochemical composition of placentas and fermented cocoa were presented in Table 1. The moisture level is very high in the fresh placenta (80.55 ± 0.42%) but also in the fermented (88.89 ± 1.49%). These high moisture levels indicate that fermented and

fresh placentas are perishable products, with conservation problems due to their hygroscopicity. These placentas are also favorable environments for the proliferation of pathogens, molds and yeasts (Aryee *et al*., 2006). High moisture content may promote microbial growth and enzymatic activity that accelerates the deterioration of this cocoa by-product. It is therefore necessary to lower the moisture content to 12% or even 7% depending on the possible subsequent uses for a longer storage period (Colas, 1998). These moisture levels obtained during this work are similar to those obtained in previous studies which are respectively 80 % (Gilet, 2006) and 82 to 87% (Hamdouche, 2015) for cocoa pulp.

The pH of the fresh cocoa placenta (4.7±0.14) and fermented (3.66 ±0.26) is acidic. The acidic pH of fresh and fermented placentas may be due to the presence of organic acids such as citric acid and acetic acid in fresh juice and cocoa placenta (Anvoh *et al*., 2009). The pH values found in fresh and fermented placentas corroborate those obtained by Quimbita *et al.* (2013) on the placenta (pH 4.41) and the juice (pH 3.25) of cocoa.

Fermentation significantly decreases the levels of total sugars and reducing sugars in cocoa placenta. In fact, the levels decrease from 13.27 ± 1.24% to 6.71 ± 0.11% for total sugars and from 8.68 ± 0.07% to 4.92 ± 0.02% for reducing sugars. This decrease in sugar levels can be due to the use of these sugars by bacteria and yeasts during the fermentation of the cocoa placenta. The total sugar content obtained in the fresh placenta are close to those obtained by Koné (2000) for fresh juice and cocoa pulp (10 to 15%), Barel (2009) for fresh cocoa mucilage 12% and Quimbita *et al.* (2013) for cocoa placenta (12.4%).

With regard to fiber, the rate in fresh placenta (30.66±3.40 %) is lower than fermented (42.66 ±2.05%). Fibers are the main constituents of the placenta after water. They could play a very indispensable role in the prevention of certain diseases such as constipation, appendicitis and colon cancer. Indeed, the fibers present in a diet facilitate the intestinal transit (Daverios, 2005). The increase in fibers levels in fermented cocoa placenta may be due to the degradation by microorganisms of other polysaccharides in addition to fibers (Lestienne, 2004). The recommended nutritional intake of fibers by food is 25-30 g / day according to AFSSA (2009). The fibers content in the cocoa placenta shows that it can sufficiently cover this daily need.

The lipids contents of fresh (0.46±0.09%) and fermented (1.73±0.19%) cocoa placentas are low despite a slight increase after fermentation. According to FAO (2003) fruit juices or pulp are generally low in fat. The lipid contents obtained corroborate those reported by Quimbita *et al*. (2013) on placenta (0.21%) and cocoa juice (0.17%).

The protein level increases from the fresh state (5.12%) to the fermented state (8.4%). Fermentation thus favored their bioavailability by destruction of membrane cells and enzymatic activity (Iga-Iga, 2008). The placenta could be useful for the formulation of animal feed (pork, chicken, fish, etc.) and for human. However, these values are much lower than those of unfermented (21.6 %) and fermented (18.8%) cocoa beans (Koné 2000, Gilet 2006, Hamdouche 2015) in foods such as soybean (37.6%), cowpea (24.3%), akpi (24.75%) (N'dri, 2010). In contrast, the level of protein in fermented placenta (8.4%) is similar to that of corn (9.2%) and rice (7.8%) (Lestienne, 2004).

The ash content in the fermented placenta (10.37±0.54%) increased slightly relative to the fresh (9.34±0.89%).The ash content of the cocoa placenta shows that it could be an excellent source of minerals. However, this content is lower than those obtained by Anno (2016) in wild mushrooms from central Côte d'Ivoire (around 14%). The high ash content reported in the cocoa placenta may be due to high levels of minerals such as potassium, phosphorus, calcium and magnesium in both types of placentas (fresh and fermented).

Table 1: Physicochemical composition of the cocoa placenta

Parameters (%)	Cocoa placenta	
	Fresh	Fermented
Moisture	80.55±0.42[a]	88.89±1.49[b]
pH	4.7±0.14[b]	3.66±0.26[a]
Totals sugars	13.27±1.24[b]	6.71±0.11[a]
Reducing sugars	8.68±0.07[b]	4.92±0.02[a]
Fibers	30.66±3.40[a]	42.66±2.05[b]
Lipids	0.46±0.09[a]	1.73±0.19[b]
Proteins	5.12±0.02[a]	8.4±0.13[b]
Ash	9.34±0.89[a]	10.37±0.54[b]

Tests: n = 3; the means ± standard deviation, assigned different letters on the same line are significantly different at p <0.05 according to Duncan's test.

The proportions in mineral contents of the two powders of cocoa placenta were shown in Table 2. The values of the mineral levels of the cocoa placenta showed that after fermentation the rates increased for K, P, Mg and Ca, except Na. In fact, the potassium (K) level increased from 1945.52 ± 0.71 mg / 100 g to 3405.51 ± 2.21 mg / 100 g. Magnesium (Mg) increased from 128.89 ± 0.99 mg / 100 g to 212.58 ± 1.03 mg / 100 g. The phosphorus (P) level increased from 154.11 ± 2.85 mg / 100 g to 173.18 ± 0.65 mg / 100 g) and finally the calcium (Ca) level increased by 92.47 ± 1 , 42 mg / 100 g to 122.37 ± 0.81 mg / 100 g. The mineral composition of the cocoa placenta has macroelements (K, P, Mg and Ca) that have high levels.

The daily needs of the body in mineral macroelements: for magnesium (Mg), this value is 360 mg for an adult woman and 420 mg for an adult man. As for potassium (K), this daily requirement is between 2 and 4 g. And finally, the daily requirement for calcium (Ca) is in the range 500 mg to 1000 mg (AFSSA, 2009).

The values of the minerals obtained in this work show that the regular consumption of the cocoa placenta could fill these daily needs which are important for the good functioning of the organism. Minerals play a vital role in animal and human health and also improve their productivity (Daverio, 2005; Burillard *et al.*, 2016). Thus, mineral deficiencies can cause disorders in animals that can even be lethal. The main function of calcium and phosphorus is the formation and maintenance of the skeleton and teeth. The skeleton uses about 99% of calcium and 80% of body phosphorus (Butler, 1989). Phosphorus deficiency would result in infertility in animals and calcium deficiency by stunting (Aryee, 2006).

In addition, the cocoa placenta contains trace elements such as sodium, iron and zinc (Table 2).The value of the sodium (Na) content in the cocoa placenta (9.34 ± 0.71 mg / 100 g) in the fresh state hardly changes in the fermented state (9.33 ± 0.81 mg / 100 g). Zn and Cu levels increased after fermentation. Zinc (Zn) decreased from 1.87 ± 0.27 mg / 100 g to 21.78 ± 1.08 mg / 100 g. The copper (Cu) content increased from 3.74 ± 0.45 mg / 100 g to 6.22 ± 0.32 mg / 100 g after fermentation. In contrast, iron (Fe) decreased after fermentation (fresh state 2.80 ± 0.27 mg / 100 g, after fermentation 1.04 ± 0.50 mg / 100 g). Finally, virtually trace level manganese (0.93 ± 0.18 mg / 100 g) in fresh cocoa placenta is undetected (UD) when fermented.

These trace elements participate in metabolic reactions for good growth.

Table 2: Mineral contents of f fresh and fermented cocoa placenta

Minerals content	Cocoa placenta	
	Fresh (g/100 g)	Fermented (g/100 g)
Mg	128.89±0.99[a]	212.58±1.03[b]
K	1945.52±0.71[a]	3405.51±2.21[b]
P	154.11±2.85[a]	173.18±0.65[b]
Ca	92.47±1.42[a]	122.37±0.81[b]
Na	9.34±0.71[a]	9.33±0.81[a]
Zn	1.87±0.27[a]	21.78±1.08[b]
Cu	3.74±0.45[a]	6.22±0.32[b]
Mn	0.93±0.18[a]	UD
Fe	2.80±0.27[b]	1.04±0.50[a]

Tests: n = 3; the means ± standard deviation, assigned different letters on the same line are significantly different at $p < 0.05$ according to Duncan's test
UD: undetected

The daily requirements of the human body in trace elements such as iron (Fe) are 16 mg for an adult woman and 9 mg for an adult male (AFSSA, 2009). Daily consumption of foods containing cocoa placenta could cover these needs and thus prevent the risk of anemia. Indeed, iron is an essential constituent for hemoglobin and its proper functioning. It is also involved in many enzymatic reactions (Pitcholo, 1990).

Potassium and sodium are needed to maintain cellular balance and nerve transmission. Deficiencies of these elements lead to muscle cramps, loss of appetite and cardiac arrhythmia (N'dri, 2010). This high amount of potassium in the cocoa placenta could increase the use of iron (Gilet, 2006) and control hypertension (Atti, 2014). In addition, cocoa placenta could be used in fertilizer formulation given its high potassium and phosphorus content. These results clearly show that cocoa placenta powder could be added in the formulation of foods to cover mineral requirements.

The total phenols, tannins and flavonoids contents are shown in Table 3. These values showed that after fermentation of the cocoa placenta, the total phenols increased (fresh 26.06±0.14%, fermented 37.5±1.02%). In contrast, the tannins (13.68±0.12%) and flavonoids (6.00 ±0.16%) levels decreased to 10.86 ± 0.08 % and 3.05±0.04%, respectively.

A comparison of the contents of phenolic compounds from fresh placenta and fermented cocoa placenta showed that there is an increase in total phenols but a decrease in tannins and flavonoids contents after fermentation. Flavonoids and especially tannins being water soluble and thermolabile, fermentation could have led to a loss of these substances. These levels of phenolic compounds found in cocoa placenta can provide valuable data for animal nutrition, as these bioactive compounds in the diet act as antioxidants and play a role in stabilizing lipids peroxidation (Van *et al*., 1998; Gülcin *et al*., 2003). The total phenols levels obtained in this work are lower than those founded by Adingra *et al*. (2017) on the epicarp of the papaya cultivar solo 8 (65.54 ±0.39%). This lowering of tannins levels after fermentation is without major risk to the fermented placenta consumer. Tannins are responsible for the antidiarrheal activity of many traditional use plants. They are known to inhibit the growth of microorganisms (Gülcin *et al*., 2003, Arbraayah & Umi, 2013). They have antibacterial, antiviral, antiparasitic, antioxidant and anti-inflammatory properties (Leitao, 2011). Sometimes tannins are a problem for the digestibility of proteins and minerals. Indeed, a diet too rich in tannins would cause the unavailability of the proteins constituting this food. Tannins are often equated with antinutritional factors. In general,

this antinutritional effect is attributed to the tannins by their inhibitory power during the digestion of dietary proteins. However, this has only been observed in non-ruminants and not in ruminants or humans who have high levels of proline-rich protein in their saliva. These proteins have a very high affinity for tannins and their presence in saliva (70 % of proteins in humans) is considered as a defense against a diet rich in tannins as the complexes formed pass intact through the digestive tract (Butler, 1989). So a human diet relatively rich in tannins would be rather beneficial thanks to their antioxidant properties.

With regard to flavonoids, they play an important role in the protection against oxidative stress with the contribution of certain vitamins (Hung & Nhi, 2012). The levels of tannins and flavonoids in the placenta of cocoa costs are substantially equal to the values obtained by Adingra *et al.* (2017) on the epicarp of the papaya cultivar solo 8. He reported that the tannins level was (10.51± 0.93%). and that of flavonoids was (5.58±0.83%).

Levels of antinutritional factors such as phytates and oxalates are also shown in Table 3. Fermentation reduced the level of oxalates and phytates in the cocoa placenta. In fact, the level of oxalates rose from 0.6±0.02% of the fresh placenta to 0.33±0.05% in the fermented. Similarly, the level of phytates in the fresh placenta (1.23±0.02%) is reduced in the fermented (0.93±0.05%). This significant decrease in these antinutritional factors could be due to the technological treatment imposed on the placenta. Indeed, one of the methods of reducing antinutritional factors in foods apart from soaking and germination is the fermentation or combination of these methods. Knowledge of the phytates and oxalates content of a food is necessary because a high level of these antinutritional compounds can have deleterious effects on digestibility (Hung *et al.*, 2012). Phytates and oxalates form complexes with essential minerals, making minerals unavailable to the body. These low levels of antinutritional factors caused by fermentation allow a safe consumption of cocoa placenta, since the lethal dose of oxalates is between 2000 and 5000 mg of oxalates/100 g of food (Mpondo *et al.*, 2012) and that of phytates between 250-500 mg of phytates/100 g of food (Medjaoui, 2017). According to Ho *et al.* (2014) in fermented cocoa beans a level of between 7 to 8 mg/g of oxalic acid was detected. The phytates level (0.93 ± 0.05%) in fermented cocoa placenta is almost identical to that of foods such as rice (0.8-1.0 %), maize (0.8-2.2%), millet (0.2 to 1.0%), sorghum (0.7 to 1.4%) and wheat (0.3 to 1.4%) (Lestienne, 2004).

Concerning antioxidant activity, it was measured by trapping radicals of ABTS and DPPH by methanolic extracts of cocoa placenta. These values are recorded in Table 3. The value of the inhibition rate of DPPH increases in the cocoa placenta after fermentation. In the fresh state, from 9.46±0.53 µM Trolox Equivalent/g DW, it increases to 13.05±0.13 µM Trolox Equivalent/g DW. The value of the inhibition rate of the ABTS also increases from the fresh state (from 12.73±0.28 µM Trolox Equivalent/g MS) to the fermented state (13.77±0.21 µM Trolox Equivalent/g DW).

Analysis of the results of antioxidant activities by radical scavenging of DPPH and ABTS showed that the fermentation allowed an increase in the antioxidant activities of the different types of placenta studied despite the decrease in tannins and flavonoids levels. One of the reasons for the increase in antioxidant activities after fermentation is due to the increase in total phenols levels and the inhibition of oxidative enzymes by fermentation. This could contribute to the body's defense by fighting infections (Daverio, 2005; Gilet, 2006). In the end, the fermentation of the placenta improves the nutritional qualities and the health interest of this food.

Table 3: Phytochemical contents and antioxidant activity of fresh and fermented cocoa placenta

Parameters	Cocoa placenta	
	Fresh	Fermented
Polyphenols (mgGAE/100g DW)	26.06±0.14[a]	37.5±1.02[b]
Tannins (mgTAE/100 g DW)	13.68±0.12[b]	10.86±0.08[a]
Flavonoids (mgQE/100g DW)	6.0±0.16[b]	3.05±0.04[a]
Oxalates (mgOA/100g DW)	0.6±0.02[b]	0.33±0.05[a]
Phytates (mgPAE/100g DW)	1.23±0.02[b]	0.93±0.05[a]
DPPH (µMTE/ g DW)	9.46±0.53[a]	13.05±0.13[b]
ABTS (µMTE / g DW)	12.73±0.28[a]	13.77±0.21[b]

Tests: n = 3; the means ± standard deviation, assigned different letters on the same line are significantly different at p <0.05 according to Duncan's test

CONCLUSION

The aim of this study was to contribute to the valorization of a cocoa by-product, in particular the placenta. At the end of this study, it appears that fermentation influences the overall level of nutrients in the placenta. In particular, levels of proteins, lipids, ash, fibers and antioxidant activity have increased while levels of sugars, tannins, oxalates and flavonoids have decreased. Cocoa placenta could be an important additive in food products as a potential natural source of nutrients, antioxidants and bioactive compounds useful for nutrition and human and animal health. Fermentation of the fresh cocoa placenta also improved its mineral composition. Fresh and fermented cocoa placenta from physiologically mature pods in west-central Côte d'Ivoire have shown good nutritional values and therapeutic compounds, but may be subject to conservation and deterioration problems due to their high water content.

For proper use of this by-product in the food industry, this placenta should be retained and included in foods as a commodity or food additive.

REFERENCES

[1] Adingra, K.M.-D., Konan, K.H., Kouadio, E.J.P. & Tano, K. Phytochemical Properties and Proximate Composition of Papaya (*Carica papaya* L. var solo 8) Peels / *Turkish Journal of Agriculture - Food Science and Technology*, 2017, **5** (6): 676-680.

[2] AFSSA (Agence Française de Sécurité Sanitaire des Aliments). Afssa – saisine n° 2007 - 0315. Maisons – Alfort, le 3 Juillet 2009, pp.38.

[3] Anvoh, K.Y.B., Zoro, B.A. & Gnakri, D. Production and Characterization of Juice from Mucilage of Cocoa Beans and its Transformation into Marmalade. *Pakistan Journal of Nutrition*, 2009, **8**: 129-133.

[4] Anno, H.F.A., Kouadio E.J.P., Konan, K.H., Dué A. E. & Kouamé, L. P. Two widely consumed wild mushrooms from central Côte d'Ivoire: their proximate analysis, mineral composition and amino acids profile. *Annals. Food Science and Technology*, 2016, **17** (1): 139-144.

[5] AOAC (Association of Official Analytical Chemists). Official Methods of Analysis of the Association of Analytical Chemists, 17th Edition Washington, DC, USA, 2000.

[6] AOAC (Association of Official Analytical Chemists). Officiel Method of Analysis of the Association of Agricultural Chemists, Washington D.C., USA, 1995.

[7] APHA. Standard methods for examination of water and waste water, 19th ed., American Public Health Association, USA, 1995, pp. 113-11.

[8] Arbaayah, H.H. & Umi K.Y. Antioxidant properties in the oyster mushrooms (*Pleurotus spp.*) and split

gill mushroom (*Schizophyllum commune*) ethanolic extracts. *Mycosphere*, 2013, **4**: 661-673.
[9] Aryee, F.N.A., Oduro I., Ellis, W.O. & Afuakwa, J.J. The physicochemical properties of flour samples from the roots of 31 varieties of cassava. *Food Control*, 2006, **17**: 916-922.
[10] Atti I. (2014). Evaluation des activités antioxydante et antiradicalaire d'un mélange d'épices « Ras el hanout ». Université Kasdi Merbah Ouargla (Algérie). pp.91.
[11] Bainbridge, Z., Tomlins, K., Willings, K. & Vestby, A. Methods for assessing quality characteristic of non grain starch staple. Part 4 advenced methods. National ressources institute, University of Greenwich, 1996, 1: 43-79.
[12] Bamba, Y. Incorporation du coprah et des cuticules de cacao et d'arachide dans l'aliment du tilapia du Nil (*Oreohromis Niloticus*, Linné, 1758) élevé en étang : effet sur la croissance et la composition biochimique. *Agronomie Africaine*, 2017, **29** (2) : 121 - 134
[13] Barel, M. De la cabosse au cacao, Éditions QUAE. De la cabosse à la tablette. 2009, pp13.
[14] Bernfeld, P. Amylase β and α (Assay method), in methods in enzymology I, Colowick and Kaplan, Ed., Academic press, New York, 1955, pp. 149-154.
[15] Burillard, L., Daumas, V., Glaz, M., Kouyoumdjian, L., Lobrot, S., Logier, D., Mallot, N. & Marchand, C. Les fermentations alimentaires. Université de Lorraine, 2016, pp. 63.
[16] Butler, L.G. Effects of condensed tannin on animal nutrition. In: *Chemistry and Significance of Condensed Tannins*. New York, 1989, 391-402.
[17] CIRAD. Cacao chez le porc en croissance finition, 2015, 15 p, http://pigtrop.cirad.fr (Pages consultées le 22/05/2019)
[18] CNRA (2012). Centre national de recherche agronomique, Côte d'Ivoire 2013, 2012, pp. 52.
www.cnra.ci/ (Pages consultées le 15/05/2018)
[19] Colas, A. Définition de la qualité des farines pour les différentes utilisations. In, Godon B., Will M C. Les industries de première transformation des céréales. Lavoisier. Tec et Doc/Apria. Paris, 1998, pp. 679.
[20] Day, R.A. & Underwood, Quantitative analysis 5th ed., Prentice London, *Hall Publication*, 1986, 701.
[21] Daverio, S. Le chocolat dans tous ses états. Université Henri Poincare-Nancy I. 2005, pp. 163.
[22] Dubois, M., Gilles, K.A., Hamilton, J.D., Rebers, R.A. & Smith, M. Colometric method for determination of sugar and related substance. *Anal of Chimie*, 1956, **28**: 350-356.
[23] Erhart, D. & Poulain, M. De l'agro écologie à la transformation locale: la quête de l'autonomie au service des producteurs, une filière équitable en Bolivie, Artisans du monde pour un commerce équitable, 2014, pp. 60.
[24] FAO (Food Agriculture Organization). The future of food and agriculture, 2017, pp. 180. www.fao.org (Pages consultées le 19/06/2019).
[25] FAO (Food Agriculture Organization). Fruits tropicaux – leurs valeurs nutritionnelles, leur biodiversité et leur contribution à la santé et à la nutrition, 2003, pp. 41. www.fao.org (Pages consultées le 28/04/2017).
[26] Gilet, F. Le cacao: des olmèques au xxie siècle. Université de Nantes. Faculté de Pharmacie, 2006, pp. 186.
[27] Gülçin, I., Oktay, M., Kireçci, E. & Küfrevioglu, O.I. Screening of antioxidant andantimicrobial activities of anise (*Pimpella anisum* L.) seed extracts. *Food Chemistry*, 2003, **83**: 371-382.
[28] Hamdouche, Y. Discrimination des procédés de transformation post-récolte du Cacao et du Café par analyse globale de l'écologie microbienne, Montpellier Sup Agro, Collège Doctorale du Languedoc-Roussillon, 2015, pp. 319.
[29] Ho, VTT, Zhao, J. & Fleet, G. Yeasts are essential for cocoa bean fermentation. International Journal of Food Microbiology, 2014, 174: 72–87.
[30] Hung, P.V. & Nhi, N.N.Y. Nutritional composition and antioxidant capacity ofseveral edible mushrooms grown in the Southern Vietnam**.** *International Food Research Journal*, 2012, **19**: 611-615.
[31] ICCO. Cocoa supply chain advances and challenges toward the promotion of decent work: a situational analysis, 2017, pp. 67.
[32] IGA-IGA, R. Contribution à la mise au point d'aliments pour tilapia *Oreochromis niloticus* à base d'intrants locaux : cas du Gabon. Agrocampus Rennes Centre de Rennes et Institut de Recherches Agronomiques et Forestières (IRAF) (Gabon). 2008, pp. 47.
[33] Kokou, E.A. & Ngo-Samnick, E.L. Production et transformation du cacao, collection Pro-Agro, CTA et ISF, 2014, pp. 44.
[34] Koné, S. Aliments et breuvages aromatiques à partir du cacao, gate Information Service gtz, PO Box 5180, 65726 Eschborn, germany, 2000, pp. 18. www.gtz.de/gate/ (Pages consultées le 22/05/2018).
[35] Latta, M. & Eskin, M. A simple and rapid method for phytate determination. *Journal Agriculture and Food Chemistry*, 1980, **67**: 1313-1315.
[36] Leitao, C. Etude de composés à intérêts technologique et fonctionnel dans la bière. Université de Strasbourg, 2011, pp.154.
[37] Lestienne, I. Contribution à l'étude de la biodisponibilité du fer et zinc dans le grain de mil et conditions d'amélioration dans les aliments de complément. Université Montpellier II. Sciences et Techniques du Languedoc, 2004, pp. 245.
[38] Manga, L. Chaîne de valeur Cacao: De l'agriculteur au consommateur, ong agrofor Cameroun. Guide technique n°4. 2013, pp. 17.

[39] Martens, H. Le cacao un levier de développement, CTB, agence Belge de développement, 2011, pp. 48.
[40] Meda, A., Lamien, C.E., Romito, M., Millogo, J. & Nacoulma, O.G. Determination of total phenolic, flavonoid and proline contents in Burkina Faso honeys as well as their radical scavenging activity. *Food Chemistry*. 2005, **91**: 571-577.
[41] Medjaoui, B. A. Étude de l'activité antioxydante de l'extrait hydroacétonique des racines de l'Arbutus unedo de la région de Tlemcen. Université Abou Bekr Belkaid de Tlemcen (Algérie), 2017, pp.66.
[42] Mpondo; M.E., Dibong, D.S., Yemeda, C.F.L., Priso, R.J. & Ngoye, A. Les plantes à phénols utilisées par les populations de la ville de Douala. *Journal of Animal & Plant Sciences*, 2012, **15** (1): 2083-2098.
[43] Muzigwa, K, Baciza, M., Bloom, J., Van, E.C., Diemby, L.M.V., Hanlet, M. & Dessalle, F. Evaluation du potentiel du Congo (R.D.C.) en déchets agroindustriels, leur composition biochimique et l'analyse de leur valeur nutritive pour les animaux en élevage. Cas de deux espèces des poissons d'eau chaude en élevage (*Tilapia spp.* et *Clarias spp.*). Université Paris VI. 1995, pp. 54.
[44] Ngambi, J.R. (2015). Déchets solides ménagers dans la ville de Yaoundé (Cameroun) De la gestion linéaire vers une économie circulaire, Université du Maine et Université Nantes Angers Le Mans. 2015, pp. 491.
[45] N'Dri, D.Y. Potentialités nutritionnelles et antioxydantes de certaines plantes alimentaires spontanées et de quelques légumes et céréales cultivées en Côte d'Ivoire. Università degli studi di parma. Faculto di agraria, 2010, pp. 112.
[46] Oqualim. Guide de Bonnes Pratiques d'hygiène de la Nutrition Animale (GBPNA) Version Projet janvier, 2016, pp. 91.
[47] Pitcholo, A.E. Essai d'utilisation des péricarpes de cabosses de cacao *(Theobroma cacao* L.) dans l'alimentation des poulets de chair au Togo. Université Cheikh Anta DIOP – Dakar. Ecole Inter-Etats des Sciences et Médecine Vétérinaires (E. J. S. M. V). 1990, pp. 120.
[48] Quimbita, F., Rodriguez, P. & Edwin, V. Uso del exudado y placenta del cacao parala obtención de subproductos. Departamento de Ciencia de Alimentos y Biotecnología Escuela Politécnica Nacional. *Revista Tecnológica ESPOL – RTE*, 2013, **26** (1): 8-15.
[49] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay *Free Radical Biology and Medicine*, 1999, **26** (9–10): 1231-1237.
[50] Shimada, K., Fujikawa, K., Yahara, K. & Nakamura, T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricutural. Food Chemistry*, 1992, **40**: 945-948.
[51] Singleton, V. L., Orthofer, R. & Lamuela-Raventos, R.M. Analysis of total phenols and other antioxydants by means of Folhin-ciocalteu reagent. *Methods Enzymology*. 1999, **299**: 152-178.
[52] Van, A.S.A, Van, de B.G.P., Berg, D.J.V.D., Bast, A., Van, D.V.W.J. (1998). Influence of iron chelation on the antioxidant activity of flavonoids. *Biochemical Pharmacology*, 1998, **56**: 936-943.