__Original Research Article__

EVALUATION OF NUTRITIVE AND ANTIOXIDANT PROPERTIES OF COCOA PLACENTA OBTAINED FROM TRHEE COCOA (*THEOBROMA CACAO L*) VARIETIES GROWN IN LÔDJIBOUA (CÔTE D'IVOIRE)

ABSTRACT

Cocoa placenta obtained after the removal of beans is a huge cocoa farm by-product usually regarded as waste by farmers. In order to contribute to their wider utilization and valorization, cocoa placenta (forastero, criollo and national) have focused our attention. The nutritive properties of cocoa placenta were investigated and the results obtained were as follow: ash (6.00 - 9.40%), proteins (7.50 - 11,40%), lipids (2.80 - 6.60%), fibers (26.20 - 36,70%) and total sugar (9.60 - 16.10%). The mineral elements contents were high with remarkable amount of K (1063.50 - 1645.12 mg/100g), Mg (196.70 - 246.89 mg/100g), Ca (96.20 - 128.41 mg/100g) and Fe (1,45 - 8.40 mg/100g). These cocoa placenta also contained appreciable levels of polyphenols (176.90 - 301.40 gGAE/Kg), flavonoids (24.60 - 79.60 gECE/Kg) and tannins (5.90 - 21.80 g/Kg). The studied cocoa placenta antioxidant varying from 9.50 - 24.40 mmolTEq/Kg for DPPH and 22.90 - 64.30 mmolTEq/Kg for ABTS. 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.

KEYWORDS : Cocoa placenta, nutritive, antioxidant properties, forastero, criollo.

INTRODUCTION

Theobroma cacao L. is considered to be the single marketable cultivated and one of the most praize food based products on the world market among the 22 known species of theobroma genus (**Sodré, 2007).** Cocoa is probably originated from central America and have been widely cultivated in some parts of the globe. Africa (Cote d ivoire, Ghana, Cameroon and Nigeria) is the leader of cocoa beans production, covering 73%, followed by America 17%, Asia and Oceania 9,9% (**FAOSTAT, 2018; ICO, 2018**). Additionally, some recent data from International Cocoa Organazation (**ICCO, 2018**) have indicated that over 4,59 million tonnes of cocoa beans were generated in the years 2017/2018, in which 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 juice and cocoa placenta (**Vásquez et al.,2019**).

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Cocoa beans constitute approximately 20%-33% of cocoa pod weight, the remaining 67%-80% is the waste by product (**Oddoye et al.,2013**). 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 **(Cádiz-Gurrea et al.,2020; Vásquez et al., 2019**). The wastes by products are rich in organic matter, including protein 5,9%-8,4%, fiber 22,6%-35,7%, carbohydrate 64%, crude fat 1,2%-10%, pectin, phenolic compounds and inorganic elements, including Ca, K, P, Mg, Na, Zn, Fe, Mn, Cu (**Sobamiwa and Longe, 1994**). 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 (**Kone et al., 2020; Oddoye et al., 2013; Vásquez et al., 2019).**

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 **(Martínez et al.,2012).** The cocoa placenta among the by products, are also rich in organic matter, phytochemicals compounds, inorganic elements and dietary fiber **(Goude et al.,2019**). 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 Ivory cost, which is considered to be the leader of cocoa cultivation and production worldwide (**ICCO, 2018**). 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 value nutritional and antioxidants of placenta of different varieties of cocoa produced in Côte d'Ivoire.Therefore

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the aim of the present study was to evaluate the nutritive and antioxydant properties of cocoa placenta from three cocoa varieties (Forastero, criollo and national) in order to valorize cocoa placenta for its potential use in food and dietetic.

MATERIAL AND METHODS

1- Material

1.1-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 6 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.

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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.

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1.2-Chemicals

All solvents (n-hexane, petroleum ether, acetone, ethanol and methanol) were purchased from Merck. Standards used (gallic acid) and reagents (metaphosphoric acid, vanillin, Folin-Ciocalteu, DPPH) were purchased from Sigma-Aldrich. All chemicals used in the study were of analytical grade.

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2-Methods

2.1-Physicochemical analysis

Proximate analysis was performed using official methods (**AOAC, 2000**). The moisture content was determined by the difference of weight before and after drying sample (5 g) in an oven (Memmert, Germany) at 105°C until constant weight. Ash fraction was determined by

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the incineration of dry matter sample (5 g) in a muffle furnace (Pyrolabo, France) at 550°C for 12 h. The percentage residue weight was expressed as ash content. For crude fibres, 2 g of dry matter sample were weighed into separate 500 mL round bottom flasks and 50 mL of 0.25 M sulphuric acid solution was added. The mixture obtained was boiled under reflux for 30 min. Thereafter, 50 mL of 0.3 M sodium hydroxide solution was added and the mixture were boiled again under reflux for 30 min and filtered through Whatman paper. The insoluble residue was then incinerated, and weighed for the determination of crude fibres content. Proteins were determined through the Kjeldhal method and the lipid content was determined by Soxhlet extraction using hexane as solvent. Total acidity (NaOH 0.1N), and pH (pH meter) were evaluated using the method described by Oludemi et Akanbi (2013) with some modifications. Total and reducing sugar were determined by **Dubois et al. (1956)** and **Bernfeld (1955)** methods, respectively.

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The color value of all cocoa placenta samples was measured by means of a portable chromameter Minolta CR 400 Osaka Japan) according to CIE Lab color scale (L*, a*, b*) as conducted by **Keskinet al (2017**). L* stand for color brithness, a* with negative and positive values for greenness and redness respectively, b* with negative and positive values for blueness and yellowness respectively. Color evaluation wos performed in 10 determination (n=100).

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2.2-Phytochemical composition analysis

Polyphenols content was determined using the method reported by **singleton et al. (1999).** 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.

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The total flavonoids content was evaluated using the method reported by **meda et al. (2005).** Briefly, 0,5 mL of the methanolic extract was mixed with 0,5 mL methanol, 0,5 mL of $AlCl_3$ (10%, w/v), 0,5 mL of potassium acetate (1 M) and 2 mL of distilled water. The mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was

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measured at 415 nm by using a spectrophotometer (PG, Instruments, England). The total flavonoids were determined using a calibration curve of quercetin (0,1 mg/mL) as standard.

Tannins of samples were quantified according to **Baindridge et al. (1996).** For this, 1 mL of the methanolic extract was mixed with 5 mL of vanillin reagent and the mixture was allowed to incubate at ambient temperature for 30 min. Thereafter, the absorbance was read at 500 nm by using a spectrophotometer (PG Instruments, England). Tannins content of samples was estimated using a calibration curve of tannic acid (2 mg/mL) as standard.

For the evaluation of antioxidant capacities of the samples, different assays were performed using the methods described by **Kesen et al. (2013)**. These were: DPPH assay, which measures the electron donation ability of the sample by bleaching of purple- colored solution of 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical. The dilution of each cocoa placenta samples was prepared in ethanol/water (v/v). An aliquot of 0.1 mL of diluted sample was added to 3.9 mL of DPPH solution in methanol (6.10^{-5}M). The mixture was shaken vigorously and left standing at room temperature for 30 min and the absorbance was measured at 515 nm using a UV–Visible spectrophotometer (Schimadzu-1201, Japan). ABTS assay was evaluated based on decolonization of radical cation of 2.20 -azino bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The ABTS radical cation was made by the reaction of 7 mM ABTS with 2.54 mM potassium persulfate, after incubation at room temperature for 12–16 h. Prior to the assay, the ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. A volume of 3.9 mL of the diluted ABTS solution was added to 0.1 mL of each sample and stand at room temperature for 30 min and immediately measured at 734 nm using a UV–Visible spectrophotometer (Schimadzu-1201, Japan). Trolox was used as standard for the calibration curve. Measurements were made in triplicate.

2.3-Mineral analysis

The dried powdered samples (5 g) were burned to ashes in a muffle furnace (Pyrolabo, France). The ashes obtained were dissolved in 10 mL of HCl/HNO3 and transferred into 100 mL flasks and the volume was made up using deionized water. The mineral composition of each sample was determined using an Agilent 7500c inductively coupled argon plasma mass spectrometer (ICP-MS) method (**CEAEQ, 2013**). Calibrations were performed using external standards prepared from a 1000 ppm single stock solution made up with 2% nitric acid.

2.4-Anti-nutritional analysis

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The titration method as described by **Day and Underwood (1986)** was performed. One (1 g) of dried powdered sample was weighed into 100 mL conical flask. A quantity of 75 mL of sulphuric acid (3 M) was added and stirred for 1 h with a magnetic stirrer. The mixture was filtered and 25 mL of the filtrate was titrated while hot against $KMnO_4$ solution (0,05 M) to the end point.

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The colorimetric method **(Latta and Eskin, 1980)**was used for the determination of phytates content. A quantity (1 g) of dried powdered sample was mixed with 20 mL of hydrochloric acid (0.65 N) and stirred for 12 h with a magnetic. The mixture was centrifuged at 12000 rpm for 40 min. An aliquot (0.5 mL) of supernatant was added with 3 mL of Wade's reagent. The reaction mixture was incubated for 15 min and absorbance was measured at 490 nm by using a spectrophotometer (PG Instruments, England). Phytates content was estimated using a calibration curve of sodium phytate (10 mg/mL) as standard.

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2.5-Statistical analysis

All the analyses were performed in triplicate and data were analyzed using EXCELL and STATISTICA 7.1 (StatSoft). Values were expressed as means ± standard deviation.

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RESULTS AND DISCUSSION

The main physico-chemical composition of cocoa placenta obtained from different cocoa varieties were evaluated in order to fully characterize this by product. As it can be shown in the table 1, an important quantity of fibers, proteins, ash and low dry matter were determined in the placenta of the three cocoa varieties. National placenta samples were found with higher amount of fibers (36.18%), proteins (11.40%), reducing sugar (9.68 g/100g) and total sugar (16.12g/100), forastero was dominated with lipid (6.6%) and low acidity while criollo placenta samples were concentrated in Ash (9.4%) and dry matter (24.17%). The pH of the different varieties placenta (3.8 - 5.6) is acidic. The acidic pH of placentas may be due to the presence of organic acids such as citric acid and acetic acid during fermentation of placenta (**Anvoh et al.,2009**).With regard to fiber, the rate in national placenta (36.7 %) is high than those criollo (31.9%) and forastero (26.2%).Fibers were found as the main constituent after water in cocoa placenta, this could be due to the decomposition of other polysaccharides in cocoa placenta by the microorganisms during fermentation(**Lestienne, 2004).**Our results corroborate **Goude et al (2019)** who recorded values ranging from 30.66 to 42.66%.Consumption of the cocoa placenta studied could be beneficial for digestion, prevention of colon cancer, treatment of gastrointestinal disorders **(Saldanha,**

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1995;UICC/WHO, 2005). The protein is high in national placenta (11.4%) than those forastero (9,5%) and criollo (7.5%). Fermentation thus favored their bioavailability by destruction of membrane cells and enzymatic activity (**Iga-Iga, 2008**). The placenta could be use for the food formulation.Our recorded contents are much lower than those of **Iniaghé et al., (2009**) which recorded in leaves of the Acalypha genus dried in the shade for 2 weeks values between 13.78 ± 0.11 and 18.15 ± 0.03%.The low level of dry matter of cocoa placenta of all varieties means that they are easily perishable and vulnerable to molds and yeasts deterioration. These results are corroborate with those previously reported in placenta samples by other authors **(Goude et al., 2019).**Lipids levels ranged from 6.60% (forastero) to 2.8% (national). These low values have been highlighted by many authors who have shown that fruit and leafy vegetables are not good sources of lipids (**Ejoh et al.,1996)**. Consumption of these cocoa placenta would be beneficial in the prevention of cardiovascular diseases, cancer and cellular aging (**Kris-Etherton et al.,2002**).Significant difference were observed between the variety of cocoa placenta in all colour parameters (L, a, b) (p<0.05). Moreover, when compared cocoa placenta colors, forastero samples showed an increase L*, a* and b* values corresponding to lighter, redder and more yellow colors, respectively. National samples show darker, and less red colors (Table 1). These variation of colors could be explained by Maillard reaction during hot air drying as placenta obtained from national is rich in proteins and reducing sugars. These physico-chemical properties of cocoa placenta could be dependent on different parameters such as region of cocoa growth, drying process, fermentation types and duration.

Table 1: Proximate physicochemical composition of cocoa's placenta

Parameters	**Forastero**	**Criollo**	**National**
Dry matter (%)	19.7 ± 4.1^{b}	24.17 ± 8.2^{a}	17.34 ± 1.9^{c}
pH	3.8 ± 0.7^{c}	5.6 ± 1.4^{a}	4.12 ± 0.6^{a}
Ash (%)	6.0 ± 2.8^{c}	9.4 ± 3.3^{a}	8.35 ± 2.9^{b}
Fibers (%)	26.2 ± 6.3^{c}	31.9 ± 9.2^{b}	36.7 ± 4.8^{a}
Lipid (%)	6.6 ± 1.9^{a}	3.8 ± 0.8^{b}	2.8 ± 0.7^{b}
Proteins (%)	9.5 ± 3.4^{b}	7.5 ± 2.4^{c}	11.4 ± 2.4^{a}
Total sugar (%)	9.6 ± 2.2^{c}	10.9 ± 2.9^{b}	16.1 ± 5.6^{a}
Reducing sugar (%)	7.0 ± 1.7^{b}	5.8 ± 1.1^{c}	9.68 ± 2.3^{a}
Color			
L*	43.8 ± 1.06^{a}	35.7 ± 3.07^{b}	29.44 ± 3.2^{c}
a*	9.4 ± 1.13^{a}	6.1 ± 1.02^{b}	4.96 ± 1.4^{b}
b*	14.8 ± 2.08^{a}	9.26 ± 2.1^{b}	11.78 ± 1.04^{b}

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

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Total phenolic content, flavonoid content, antioxydant potential, oxalate, tannins, phytates and minerals measured in cocoa placenta samples are presented in Table 2. Total phenolic content (301.4 g GAE/kg), flavonoids (63.7 gECE/kg) and antioxydant activity as ABTS (64.3 mmol TEq/kg) and DPPH (24.4 mmol TEq/Kg) were more abundant in criollo placenta samples compared to national and forastero (Table 2). The results of antioxidant activities by radical scavenging of DPPH and ABTS showed that an increase in the antioxidant activities of the different types of placenta studied. 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**(Goudé et al., 2019**).The significant concentrations of these bioactive compound determined in cocoa placenta could provide valuable data for animal feed formulation and nutritionsince Phenolic compounds have numerous bioactive effects such as antioxidant, anti-inflammation, detoxification, immune protection, hormone modulation, anti-tumour, cardioprotection, antidiabetes, neuro-protection, anti-allergy and endothelial protection**(Xan et al., 2007 ; Kang et al., 2011).** Our results are higher than those presented in former studies by **Goude et al. (2019).** An increase amount of tannins (21.8 g/Kg), phytates (3.5 g/Kg) and oxalate (4.4 g/kg) were detected in cocoa placenta of forastero, while a small amount of these substances were observed in national and criollo placenta samples. This significant decrease in these antinutritional factors could be due to the fermentation imposed on the placenta. Indeed, one of the methods of reducing antinutritional factors in foods apart from soaking and germination is the fermentation (**Goudé et al.,2019**). These low levels of antinutritional factors caused by fermentation allow consumption these 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**). These low levels could be advantageous for the consumer since these compounds reduce the bioavailability of certain minerals.

Regarding the minerals composition, sodium (Na), magnesium (Mg), phosphorus (P), potasium (K), calcium (Ca), copper (Cu), iron (Fe) and zinc (Z) were detected in all cocoa placenta, except manganese (Mn) which was only detected in criollo samples as shown table 2. Minerals are reported to increase in fermented cocoa placenta when compared to unfermented cocoa placenta **(Goude et al.,2019).** Of all minerals identified, potasium (K) was quantitatively the most prevailing mineral in forastero (1645 mg/100g), criollo (1435 mg/100g), and national (1063 mg/100g). Magnesium (Mg) was the second major mineral follow by calcium (Ca) found in all cocoa placenta samples. Minerals play an essential role in

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animals and human health by improving their productivity. For instance, calcium and phosphorus are reported to participate in the formation, solidification and maintenance of animal and human skeleton and teeth. Mineral deficiencies can cause some disorders in animals and human, which can result to death. Therefore, the findings obtained in this present study could be used as a route for the incorporation of cocoa placenta in the formulation of animals products in order to fill the daily needs which are vital for the good functioning of the organisms.

Table 2: Proximate nutritional and anti-nutritional composition of cocoa's placenta

Parameters	**Forastero**	**Criollo**	**National**
ABTS (mmol TEq/kg)	22.9 ± 1.4^c	64.3 ± 3.5^a	38.4 ± 6.4^b
DPPH (mmol TEq/kg)	9.5 ± 0.5^c	24.4 ± 3.6^a	18.9 ± 2.9^b
Polyphenols (g GAE/kg)	176.9 ± 10.7^c	301.4 ± 8.4^a	224.8 ± 14.6^b
Flavonoids (gECE/kg)	24.6 ± 2.1^c	63.7 ± 7.8^b	79.6 ± 9.4^a
Oxalates (g/Kg)	4.4 ± 0.6^a	1.9 ± 0.4^b	0.8 ± 0.1^b
Phytates (g/kg)	3.5 ± 1.2^a	1.3 ± 0.3^b	1.0 ± 0.0^b
Tannins (g/kg)	21.8 ± 4.3^a	8.4 ± 1.5^b	5.9 ± 0.9^c
Mineral (mg/100g)			
Na	12.36 ± 2.5^b	9.5 ± 1.3^c	14.9 ± 6.2^a
Mg	246.89 ± 17.3^a	224.1 ± 9.4^b	196.7 ± 12.4^c
P	6.12 ± 1.4^b	3.2 ± 0.5^c	11.3 ± 3.6^a
K	1645.12 ± 21.3^a	1435.1 ± 36.1^b	1063.5 ± 14.3^c
Ca	128.41 ± 6.2^a	96.2 ± 10.3^b	104.8 ± 7.2^c
Mn	<LOD	1.4 ± 0.6	<LOD
Cu	3.89 ± 1.2^b	7.9 ± 3.7^a	3.0 ± 0.4^b
Fe	1.45 ± 0.1^c	8.4 ± 2.8^a	3.8 ± 0.9^b
Zn	2.17 ± 0.4^a	1.5 ± 0.2^c	<LOD

* LOD: limit of detection; values are means and standard deviations (mean (SD)) of three measurements for color analysis and three replications for other experiments. Different lowercase letters on the numbers in the same line represent significant differences ($p < 0.05$).

CONCLUSION

The present study was conducted to elucidate, nutritive composition and antioxidants of cocoa placenta obtained from three different cocoa cultivars, including forastero, criollo and national. Based on the results obtained, cocoa placenta possesses a substantial amount of nutritif and phenolic compounds. The highest amount of phenolics and antioxydant capacity was determined in criollo. 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.

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