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Adding Value to Cacao Pod Husks as a Potential Antioxidant-Dietary Fiber Source

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Abstract Côte d'Ivoire (Ivory Coast) is the world's largest cocoa producer with about 1.2-1.6 million tons per year. This co-generates approximately ten times of fresh cacao pod husks, which are hitherto left unutilized to decompose in plantations. This study aims at evaluating the cacao pod husks potential for antioxidant-dietary fiber compounds. Cacao pod husk product was used for the extraction of dietary fiber and phenolic compounds. The results showed that the cacao pod husk product contained ~ 60.0% of total dietary fiber, of which non-starchy polysaccharides accounted for > 70.0%, and a total phenolic content of ~69.0 mg Gallic acid equivalent/g, thereby indicating that it was an antioxidant dietary fiber-rich product. It also exhibited interesting antioxidant properties, as judged by 2,2-Diphenyl-1-picrylhydrazyl (85.0% inhibition percentage and EC_{50} = 25.0 g/g), 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (52.0 µmol Trolox equivalent/g), and Ferric reducing antioxidant power (130.0 µmol Trolox equivalent/g) assays. The total antioxidant capacity of the cacao pod husk product was significantly higher ($P < 0.05$) than the total antioxidant capacity of fermented-and-roasted cocoa hull and kernel products. The total antioxidant capacity seemed to result from synergistic interactions among various compounds endowed with antioxidant capacity, including soluble phenolics, condensed tannins, and possibly pectic substances. Cacao pod husks therefore appeared to be a valuable source of antioxidant dietary fiber-rich food materials which may be used to significantly reduce the risk of development of miscellaneous free radical-induced diseases.

Keywords: *cacao pod husks, dietary fiber; hydration properties, polyphenols, antioxidant activity*

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1. Introduction

There is an increasing interest for searching for plant sources with high contents in natural antioxidant compounds and dietary fiber, as redeemed by the numerous scientific reports on them for several decades. Antioxidant compounds and dietary fiber have indeed shown interesting health benefit properties and protective effects against various free radical-induced pathologies. Antioxidant compounds are therefore usually counted among the protective elements that compose our first line of defence against free radical damages due to their capacity to scavenge them. Free radicals, of which reactive oxygen species (ROS) are the most threatening, play a crucial role in the pathogenesis of various human disorders, such as cancer, rheumatoid arthritis, neurodegenerative, diverticular, coronary-heart, and pulmonary diseases [1,2]. ROS are an entire class of highly reactive molecules (e.g., hydrogen peroxide, hydroxyl radical, and superoxide radical) derived from the metabolism of oxygen. They are often generated as byproducts of biological reactions or from exogenous factors. In vivo, some of the free radicals play positive roles in cell physiology; however, the balance between the production of ROS and their neutralization by radical scavenging antioxidant compounds is very delicate and if this balance turns in favour of overproduction of ROS relative to antioxidant compounds stores, normal cells start to suffer the consequences of oxidative stress [2,3]. This may seriously be harmful to human health due to occurrence of uncontrolled damages to cell membranes and DNA and various oxidation processes, thereby engendering membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations, a result of which is the possible appearance of miscellaneous pathologies as afore-mentioned. Despite its remarkable efficiency, the endogenous antioxidant system does not suffice, and humans depend on various types of antioxidant compounds that are present in diets to maintain free radical concentrations at low levels [2]. A low total antioxidant capacity (TAOC) is considered to be a risk factor for the development of disorders such as cancer and cardiovascular diseases, and therefore adequate amounts of antioxidant compounds are required for showing effective protection against free radical damages [3]. A direct relationship between the TAOC and total phenolic

content (TPC) of diverse plant food materials has been reported [1,4]. Epidemiological studies have also shown that consumption of phenolic- and dietary fiber-rich food products can effectively reduce the risk of upsurge of coronary, diverticular, and certain cancer diseases [1,6,7]. The intake of antioxidant compound-rich and dietary fiber-rich foodstuffs are therefore of a capital importance for maintaining optimum health balance and welfare. Thus, antioxidant-containing foods such as vegetables, fruits, soy products, cocoa and teas that counteract ROS are expected to be protective against cancer causation and development [8]. In this connection, various studies providing evidence for the potential for preventive effects of cocoa polyphenols and other antioxidant compounds against cancer development have recently been largely reviewed [6,8]. By contrast, work on the antioxidant-dietary fiber (ADF) content of cacao pod husks remains rather scarce hitherto, and yet cacao pod husks generally account for more than 50% of fruit fresh weight. Moreover, for each ton of dried beans produced, about 16 tons (on a fresh weight basis) of cacao pod husks are co-generated, thereby representing a serious disposal problem [9]. In traditional everlasting practices, the cacao pod husks are left to decompose in plantations, thus producing foul odors and becoming a quantitatively important source of plant disease inoculum such as black pod rot [10]. With a production of about 1.2-1.6 million tons per year, Côte d'Ivoire is currently the world's leading producer of cocoa beans. This cogenerates a huge amount (~12-16 million tons) of fresh cacao pod husks, to which no value has been added to date. The scope of this study was to evaluate the total phenolic and dietary fiber contents and antioxidant capacity of cacao pod husks for possible use as a supplement product for these valuable plant-derived bioactive components.

2. Materials and Methods

2.1. Raw Plant Materials

Mature ripe cacao pods were harvested, in three batches, from an experimental cacao plantation of new hybrids (CNRA, Abidjan, Côte d'Ivoire) with a high productivity, derived from the commonly cultivated Forastero, in a town (Bacon, Adzopé) located in the southeastern region of Côte d'Ivoire. Random selection of fruits was carried out among the different batches and selected fruits were dry-cleaned from impurities (such as dust) using vacuum cleaner and the average mass of pods was determined by weighing. Each pod was cut into two parts to remove the pulpy seeds and mucilage. The husks were then thoroughly washed with water several times to remove residual mucilage, grossly chopped to pieces with the help of a stainless steel knife, roughly minced in a Kenwood apparatus, soaked in 2% aqueous sodium hypochlorite, for preservation and prevention of possible browning reaction, and finally oven-dried at 40-45°C to constant weight. Dried cacao pod husks were roughly ground in a hammer mill (Model 912, Winona Attrition Mill Co., Winona, MN) to pass through a 12 mm size screen and the resulting material, henceforth referred to as cacao pod husk product (CPHP), was kept under moisture-free conditions pending analysis. For certain (compositional and physicochemical) analyses, however, samples of CPHP were finely ground to pass through 60-mesh (# 0.25 mm) size sifters.

For comparison purposes, kindly supplied fermented-and-roasted cocoa hull (CHPFR) and cocoa kernel (CKPFR) products, in three samples for each, from the same hybrid cultivar, by the Abidjan's Great Mill (GMA) Plant (Les Grands Moulins d'Abidjan, Abidjan, Côte d'Ivoire), were used. All the experiments were carried out in three independent runs.

2.2. Evaluation of the Physicochemical Properties of Cacao Byproducts

The swelling capacity (SwC), water retention capacity (WRC), and oil retention capacity (ORC) of the different cacao materials were determined as fully described elsewhere [7,11]. SwC is a measure of the ratio of the volume occupied by the sample immersed in an excess of water, after an overnight equilibration, to the sample actual weight. WRC is a measure of the amount of water retained by a known weight of fiber, following application of an external force (centrifugation or pressure) under the conditions used and is preferred to either water-holding capacity or water-binding capacity. ORC, also known as fat retention (or absorption) capacity, is a measure of the ratio of the quantity of oil held up (from the amount added), following application of an external force (centrifugation or pressure), to the initial dry weight of sample and is preferred to oil holding capacity.

2.3. Preparation of Soluble Phenolic Compounds from Cacao Byproducts

To extract soluble substances including polyphenols and soluble solids (free sugars), CPHP, CHPFR, and CKPFR were submitted to four different sequential treatments in terms of the solvent kind, firstly by hot ethanol-water extraction, followed by acetone-water extraction at room temperature, 100% distilled water extraction at 37°C, and finally by hot acidified butanol (Porter's reagent) extraction as follows:

Dry raw materials (1 g) were treated in boiling 80% (v/v) ethanol for 20 min (solid/liquid ratio 1: 25 w/v), cooled to room temperature, centrifuged (3000*g*, 20 min) and filtered through G-3 sintered glass, and then on nylon cloth (0.45 µm). Insoluble solids were repeatedly washed with 60% (v/v) acidified aqueous ethanol (5 mL of concentrated hydrochloric acid/L) at room temperature and the washing was added to filtrate until it contained no free sugars, as judged by a negative response with a sugar assay [12]. A final washing was then done with 95-100% ethanol. The insoluble solids (residues left) were treated twice with 25 mL of 60% (v/v) aqueous acetone at room temperature for 60 min, centrifuged (3000*g*, 20 min) and filtered through G-3 sintered glass and on nylon cloth (0.45 µm). The ethanolic and acetonic fractions were pooled after concentration, by rotary-evaporation at 40°C, to desired quantity. Aliquots were taken and kept at -20°C pending analysis for soluble sugars. The organic solvent insoluble residues were treated two-times with 25 mL of 100% distilled water at 37°C for 60 min and processed as above (to obtain a pure water-soluble fraction). All the

three fractions were combined, freeze-dried and considered to be a fraction of soluble phenolics. The water-insoluble residues were finally treated two-fold with 25 mL of acidified (12 N HCl-) butanol (5:95, v/v) (Porter's reagent) at 100°C for 180 min and managed as above to solubilise depolymerised condensed tannins. The latter were spectrophotometrically analysed at 550 nm [13]. All the experiments were performed in three independent runs.

2.4. Compositional Analyses of Cacao Byproducts

Moisture, fat, protein, and ash contents were determined by a conventional method as previously done [7]. Total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) of defatted samples of CPHP, CHPFR, and CHPFR were measured by the enzymatic-gravimetric AOAC (Association of Official Analytical Chemists) method using MES-Tris buffer [14] and as the sum of non-starchy polysaccharides (NSP) and "Klason lignin" by the Saeman hydrolysis method, according to Figure 1 [7].

Total soluble sugar, sucrose and fructose were colorimetrically analysed [15,16] on a UV-Vis Perkin-Elmer Lambda 3 Double Beam Spectrophotometer (PerkinElmer Corp., Norwalk, CT). Alternatively, sucrose was evaluated by difference in reducing sugars [17] before and after enzymatic treatment with by β-D-fructofuranosidase (EC 3.2.1.26; Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). Galactose, glucose, mannose, and fucose released were estimated as described elsewhere [18]. Glucose was also analysed by the rapid glucose oxidase (EC 1.1.3.4; Sigma-Aldrich Co., St. Louis, MO)-Fenton's reaction method [19]. Arabinose and rhamnose were spectrophotometrically quantified using Megazyme assay kits (Megazyme International Ireland Ldt., Bray, Co. Wicklow, Ireland). Xylose was colorimetrically assessed [20] and uronic (galacturonic and glucuronic) acids were estimated by a modified sulfamate-meta-hydroxydiphenylassay [21]. The monosaccharide constituents of the NSP present within the soluble fractions were released by a straightforward acid-hydrolysis with 1 mol.L^{-1} H_2SO_4 (100°C, 3 h), and those of NSP within insoluble (cell wall) materials were released by a two-stage Saeman acid-hydrolysis, first by 12 M H_2SO_4 (23°C, 1 h) followed by dilution to 1 M H_2SO_4 and incubation at 100°C for 3 h [7,22].

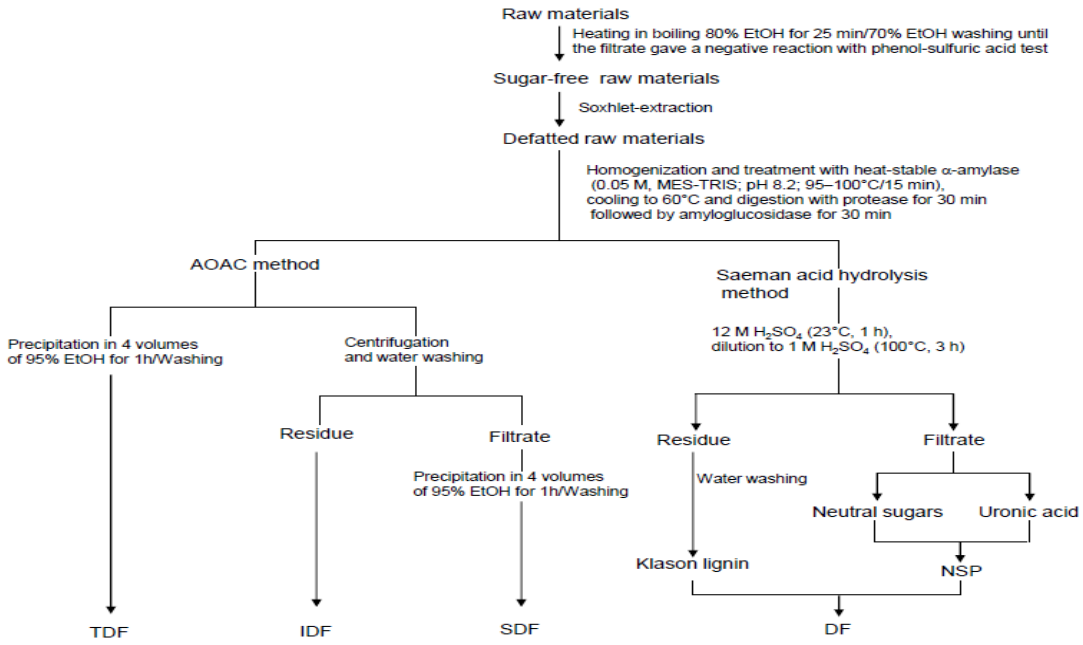

Figure 1. Scheme of isolation and characterization of dietary fiber-rich materials from cacao by-products

Elemental minerals (K, P, Ca, Mg, Fe, and Na) were analysed by a flame atomic absorption spectrometry [23] using an Aanalyst 300 spectrophotometer (Perkin-Elmer Corp., Norwalk, CT). Analyses were carried out in triplicates for each sample used.

2.5. Determination of the Total Phenolic Content and Total Antioxidant Capacity of Cacao Byproducts

2.5.1. The Total Phenolic Content

The total phenolic content (TPC) of extracts was determined by a colorimetric Folin-Ciocalteu reagent (Sigma-Aldrich Co., St. Louis, MO) assay [24]. Briefly, 0.1 mL of sample (extract) was added to test tube, followed by addition of 6 mL of distilled water and 0.5 mL of the Folin-Ciocalteu reagent. After mixing and resting for 3 min, 1.5 mL of 20% (w/v) sodium carbonate was added and the test tube was adjusted to 10 mL and thoroughly mixed. The increase in absorbance of the colour generated, after 2 h at room temperature, was spectrophotometrically determined at 760 nm against an appropriate control (or "blank"). Gallic acid (25-500 µM) was used as standard and the results were expressed as gallic acid equivalents (mg GAE)/g dried weight of sample. Analyses were carried out in triplicates for each sample used.

2.5.2. DPPH Scavenging Antioxidant Assay

The total antioxidant capacity (TAOC) of samples was first appraised for its ability to scavenge the stable free radical 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$; Sigma-Aldrich Co., St. Louis, MO) in two ways:

For a fixed point method, as a common practice in most laboratories, 0.2 mL ethanolic solution of antioxidant-containing samples (0.5 mg/mL) were added to 0.8 mL of a 50 mM Tris-HCl buffer (pH 7.5) and vortex-mixed, after which 1 mL of a 0.1 mM ethanolic solution of $DPPH^{\bullet}$ was added. The reaction mixture was vortex-mixed thoroughly and left in the dark at 23°C for 30 min. The decrease in absorbance of the mixture, at 23°C, was spectrophotometrically measured at 515 nm. Trolox (0.1-1.0 mM) was utilised as antioxidant standard. Antioxidant activity or inhibition percentage (IP), defined as the ability of the sample to scavenge $DPPH^{\bullet}$, was calculated as follows:

$$IP(\%) = \left[\left(Abs_{control} - Abs_{sample}\right)\right] / \left(Abs_{control}\right)] \times 100$$

where $Abs_{control}$ and Abs_{sample} are the absorbances of $DPPH^{\bullet}$in the absence and presence of the antioxidant-containing sample, respectively.

For a steady sate saturation method [25], 0.1 mL of an ethanolic solution of sample was added to 3.9 mL of a 24 mg/L of ethanolic solution of $DPPH^{\bullet}$. The decrease in absorbance was determined at 515 nm at different time intervals until the reaction reached a steady state (a plateau-like curve). The initial concentration of $DPPH^{\bullet}$ in the reaction medium was calculated from a calibration curve, as obtained by linear regression (R^2 = 0.989), and the percentage of remaining $DPPH^{\bullet}$ at steady state ($\%DPPH^{\bullet}_{Rem}$) was calculated and plotted against the sample concentration. The antiradical activity was determined in terms of the efficient concentration (EC_{50} (g dry weight sample/g $DPPH^{\bullet}$)), viz. the amount of antioxidant-containing sample required for 50% decrease in the initial $DPPH^{\bullet}$ concentration. Analyses were carried out in triplicates for each sample used.

2.5.3. ABTS Scavenging Antioxidant Assay

The TAOC of samples was also evaluated by its capability to scavenge ABTS (Sigma-Aldrich Co., St. Louis, MO) radical [26]. Briefly, 7 mM ABTS stock solution was prepared in water. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting equal quantities of ABTS stock solution with 2.5 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 15 h before utilisation. The generated $ABTS^{\bullet+}$ solution (1 mL) was then diluted with ethanol (60 mL) to an initial absorbance of 0.802 ± 0.01, read at 734 nm, and the reagent obtained was equilibrated at 30°C. After adding 0.9 mL of the latter $ABTS^{\bullet+}$ solution to 0.1 mL of antioxidant-containing samples in test tubes and vortex-mixing, the decrease in absorbance of the mixtures, at 30°C, was measured at 734 nm from 1 min up to 7 min against an appropriate control (or "blank"). Trolox (Sigma-Aldrich Co., St. Louis, MO), at a concentration in the range of 0.1-1.0 mM, was used as standard and the results were expressed as µmol of Trolox equivalent (µmol TE)/g dried weight of sample. Analyses were carried out in triplicates for each sample used.

2.5.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The TAOC of samples was finally evaluated through its ability to reduce ferric (Fe^{3+}) cation to ferrous (Fe^{2+}) cation [27]. The stock solutions encompassed 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (Sigma-Aldrich Co., St. Louis, MO) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working FRAP reagent was prepared by mixing acetate buffer, TPTZ, and $FeCl_3{\cdot}6H_2O$ solutions in a ratio of 10:1:1 (v/v/v), respectively; and was warmed up to 37°C before use. Antioxidant-containing samples (0.1 mL) were added to 0.9 mL of the FRAP solution in test tubes, and the mixtures were vortex-mixed and allowed to react for 30 min in the dark. The increase in absorbance of the mixtures, at 37°C, was monitored at 593 nm from 4 up to 30 min against an appropriate control (or "blank"). Trolox (0.1-1.0 mM) was used for calibration and the results were expressed as µmol TE/g dried weight of sample.

2.6. Statistical Analysis

All the data were statistically appraised by a single-factor analysis of variance (ANOVA), followed by the Bonferroni's posthoc test for multiple comparisons, whenever applicable, using a GraphPad Prism V.3 software (GraphPad software Inc., San Diego, CA). Means of different treatments were significantly discriminated at $P = .05$. Possible correlation existing between TPC and TAOC of samples were analyzed by the widely used Pearson's test.

3. Results and Discussion

3.1. Physical Composition of Cacao Pods

The average fresh weight of mature ripe cacao pods was 516.7 ± 1.9 g (three measurements), in agreement with that (474.77-500 g) reported elsewhere [9]. The average fresh weight of beans was 121.8 ± 1.7 g. The produced husks accounted for 70.6 ± 2.4% (w/w) of the fresh pods and were mainly composed of moisture (84.6 ± 2.2%). These results are in fairly good accord with literature data, specifying 74-76% and 90% for the amount of husks and their moisture content, respectively [9]. Thus, the dried husks were estimated at ~11.0% of the fresh pod weight.

3.2. Chemical Composition of Cacao Pod Husk Product (CPHP)

The results of the proximate analyses of CPHP are shown in Table 1.

CPHP was mainly composed of NSP (42.0%), followed by soluble sugars (13.0%), proteins (9.0%) and ash (8.0%). The amount of reducing sugars (mainly fructose with some glucose) represented about 64.0% of the total soluble sugar assimilated to sucrose. Soluble phenolics were about 7.0% of the CPHP dry weight. Various elemental minerals were detected in CPHP, four of which (K, P, Ca and Mg) were quantitatively important (0.5-6.0%)). The abundance of NSP and soluble phenolics in CPHP suggested that it could be a good ADF source. Moreover, the presence, in notable quantities, of essential minerals such as K, Ca, and Mg, which are required for maintaining vital functions in living human cells, makes it a potential source of those elements. In comparison with CHPFR and CKPFR, CPHP had a similar NSP content to CHPFR, but appeared to be richer in these components than CKPFR. It contained less proteins than CHPFR and

CKPFR, but more lipids than the former. It is, however, remarkable that more than 50% of CKPFR was composed of lipids, in agreement with previous studies [28,29]. This indicated that fermented-and-roasted cocoa kernel product was a fat-rich source. Reducing sugars (fructose and glucose) were found to be the major soluble sugars in CPHP, whereas non-reducing sugars (assimilated to sucrose) were more abundant in CHPFR and CKPFR. This discrepancy was probably due to the fact that during roasting, reducing sugars, in contrast to non-reducing sugars, were heavily destroyed via interactions with aminoacids (Maillard reactions) to form other compounds (Maillard products) as has been suggested elsewhere [29].

In general, the ash, fat, and protein contents of CPHP, CHPFR and CKPFR are in line with those reported, by other workers, for cocoa hull and kernel products: 6.7-8.42%, 1.5-2.34%, and 4.21-8.6%, respectively [5,9]. By contrast, the soluble sugar content of CPHP (13.0%) was substantially lower than those (19.2-29.04%) found for similar cocoa products in previous studies [5,9].

3.3. Dietary Fiber Composition of CPHP

The rather high NSP content (42.0%) of CPHP presaged for good dietary fiber potentials. The results of analysis of the dietary fiber composition are presented in Table 2. The amount of TDF in CPHP was 59.0%, including 11.0% SDF and 48.0% IDF, as determined by the AOAC method. This showed that CPHP was a fiber-rich source which might be used to increase dietary fiber intake of fiber-poor diets, especially in Western countries. Dietary guidelines, indeed, recommended a minimum daily intake of dietary fiber of 25 g (equivalent to 12.5 g dietary fiber per 1000 calories consumed), which is considerably higher than the estimated intakes in Western countries [30]. The ratio of IDF to SDF was about 4.20, suggesting that CPHP may be suitable for improving bowel transit time [7]. The dietary fiber composition of CPHP was similar to that of CHPFR, but significantly different ($P < 0.05$) from that of CKPFR.

Table 1. Chemical composition (mg/g) of CPHP, CHPFR, and CKPFR

Proximate composition (mg/g)	CPHP	CHPFR	CKPFR
Moisture	84.5 ± 3.1	76.1 ± 3.8	65.2 ± 1.1
Ash	79.2 ± 3.9a	80.3 ± 7.6a	37.1 ± 0.8b
Fat	22.7 ± 2.5a	62.8 ± 4.7b	565.4 ± 13.2c
Proteins (N × 6.25)	89.1 ± 2.9a	153.5 ± 4.6b	129.6 ± 3.1b
NSP	420.7 ± 2.3a	452.8 ± 1.5a	141.9 ± 1.1b
Total soluble sugars	131.8 ± 1.6a	74.2 ± 3.3b	19.4 ± 1.5c
Reducing sugars	84.3 ± 4.1a	18.9 ± 3.2ab	2.1 ± 0.1b
Soluble phenolics (mg GAE/g)	68.9 ± 5.6a	26.7 ± 4.5b	22.6 ± 0.9b
Mineral elements (mg/g)			
K	58.2 ± 3.7a	21.7 ± 0.7b	16.9 ± 0.3b
P	7.1 ± 0.9	5.9 ± 0.2	6.5 ± 0.1
Ca	6.1 ± 0.8a	4.2 ± 0.1a	2.1 ± 0.2b
Mg	4.9 ± 0.6a	2.5 ± 0.1b	5.2 ± 0.1a
Fe	0.35 ± 0.05	0.45 ± 0.01	0.14 ± 0.01
Na	0.16 ± 0.02	0.14 ± 0.01	0.11 ± 0.02

Data are expressed as mean ± SD (n = 3). Values in the same line with different letters are significantly different at $P = .05$ according to the Bonferroni's test.
CPHP: ("fresh") cacao pod husk product; CHPFR: fermented-and-roasted cocoa hull product; CKPFR: fermented-and-roasted cocoa kernel product
GAE: gallic acid equivalent
NSP: non-starchy polysaccharides

Table 2. Dietary fibre and glycosyl residue composition (mg/g) of CPHP, CHPFR, and CKPFR

DF composition (mg/g)	CPHP	CHPFR	CKPFR
AOAC method			
SDF	114.6 ± 9.4a	74.1 ± 4.9b	72.4 ± 1.2b
IDF	478.3 ± 2.8a	523.4 ± 8.2a	125.9 ± 2.8c
TDF	590.2 ± 5.4 a	601.4 ± 5.7a	194.3 ± 1.4b
IDF/SDF ratio	4.17 ± 0.91a	7.06 ± 0.4b	1.74 ± 0.08c
Saeman hydrolysis method			
Rhamnose	32.1 ± 0.8a	29.4 ± 1.3a	2.6 ± 0.3b
Fucose	2.5 ± 0.4a	0.9 ± 0.1b	0.5 ± 0.1c
Arabinose	28.9 ± 1.6a	19.3 ± 1.2b	5.6 ± 0.1c
Xylose	46.2 ± 1.4a	23.6 ± 0.4b	5.9 ± 0.2c
Mannose	15.2 ± 0.4a	18.5 ± 0.4a	1.7 ± 0.04b
Galactose	42.3 ± 1.8a	42.7 ± 4.5a	8.2 ± 0.1b
Glucose	145.6 ± 8.4a	181.5 ± 2.8b	79.8 ± 1.2c
Glucuronic acid	11.4 ± 0.4a	15.2 ± 1.1a	4.7 ± 0.3b
Galacturonic acid	96.5 ± 1.2a	121.7 ± 2.1b	32.9 ± 1.2c
NSP	420.7 ± 2.3a	452.8 ± 1.5a	141.9 ± 1.1b
Klason lignin	193.5 ± 6.9a	159.4 ± 3.7a	59.2 ± 3.7b

Data are expressed as mean ± SD (n = 3). Values in the same line with different letters are significantly different at $P = .05$ according to the Bonferroni's test.
AOAC: Association of Official Analytical Chemists
CPHP: ("fresh") cacao pod husk product; CHPFR: fermented-and-roasted cocoa hull product; CKPFR: fermented-and-roasted cocoa kernel product
DF: dietary fiber; IDF: insoluble dietary fiber; SDF: soluble dietary fiber; TDF: total dietary fiber
NSP: non-starchy polysaccharides

Furthermore, the Saeman method revealed that dietary fiber was composed of 42.0% NSP and 19.0% "Klason lignin", indicating that polysaccharides were the dominant fiber components of CPHP. The composition in glycosyl residues showed that glucose (~15.0%) and galacturonic acid (~10.0%) were the two major monosaccharide residues, indicating that cellulose and pectic substances were the predominant NSP in cacao pod husks. Similarly, it has also been found that these two types of polysaccharides are dominant in CHPFR and CKPFR, consistent with previous work [29,30,31]. Moreover, the detection of relatively high amounts of xylose (~4.0%) and galactose (~4.0%) along with low amounts of mannose (~2.0%) and glucuronic acid (~1.0%) suggested the presence of various non-cellulosic cross-linking heteroglycans, namely xyloglucans, galactoglucomannans, and glucuronoarabinoxylans as has previously been demonstrated for cocoa shells from West African beans (Forastero var) [31].

In comparison with other dietary fiber sources, the TDF content of CPHP (59.0%) is higher than those of citrus (35.40-36.9 %), apple (51.11%) and banana (32.9-51.9%) byproducts [32,33,34], but lower than those of coconut fiber (63.24%), pea hull (75.12%) and yellow passion fruit rind (73.5-81.9%) byproducts [7,11,33].

It is worth mentioning that the definitions of dietary fiber do not actually make a worldwide consensus. Initially, dietary fiber has been defined to be plant cell wall remnants that are not hydrolysed by human alimentary enzymes. This definition has subsequently been modified to include all the polysaccharides and lignin in the diet that are resistant to endogenous secretions of the human digestive tract. Accordingly, current definition of dietary fiber refers to NSP, resistant starch, fructan oligosaccharides, and lignin, upon which the AOAC method for the determination of TDF is based. In several countries, however, the definition of dietary fiber is strictly limited to the NSP of the plant cell wall and oligosaccharides components that are resistant to hydrolysis by human alimentary enzymes. Moreover, in the specific case of cacao-derived byproducts, such as cocoa hull (or shell) and de-hulled cocoa beans, tannin-protein complexes and Maillard reaction products, from fermentation and roasting, also appeared largely insoluble after the Saeman hydrolysis by which "Klason lignin" was determined [29,31]. Therefore, in material which contains significant amounts of these complexes, the term ''Klason lignin'' is somewhat meaningless as a measurement of actual lignin. This has significance for the estimation of dietary fiber in such materials, because lignin is included in the definition of dietary fiber whereas tannin-protein complexes and Maillard products are not [29,31]. By contrast, according to some workers [30], "Klason lignin" would encompass not only actual lignin (consisting of 3 monomeric subunits—*p*-coumaryl, guiacyl, and sinapyl-propane), but also other polyphenols (condensed tannins), resistant protein, and Maillard products, all assumed to be non-digestible constituents of plant food materials that might account for some of the physicochemical properties of dietary fiber.

Nevertheless, as regards the cacao pod husks that were used in this study, no fermentation and roasting treatments were applied to them before analysis, and therefore one can expect that no "artificial lignin-like compounds" were present in CPHP and that its "Klason lignin" corresponded to actual lignin, contrary to CHPFR and CKPFR which underwent fermentation and roasting treatments.

3.4. Physicochemical Properties of CPHP

The WRC, SwC, and ORC of CPHP were 6.5 g/g, 7.3 mL/g, and 1.9 g/g, respectively Table 3.

The WRC and ORC of CPHP were greater than those of CHPFR, although both had similar TDF contents Table 2. This may be ascribed to the presence of higher proportion of SDF in CPHP than in CHPFR, as judged by the IDF/SDF ratio values Table 2. In general, the SDF fraction of a fiber product is mainly responsible for the hydration capability. In comparison with other fiber sources, the hydration properties of CPHP are similar to those of carob pod fiber (5.53 g/g WRC), apple pulp (3.5-5.0 g/g WRC and 7.0-9.0 mL/g SwC), and yellow passion fruit rind (3.7-4.1 g/g WRC); higher than those of cellulose, pea, wheat, and carrot fiber products (0.7-3.1 g/g WRC and 0.0-7.5 mL/g); but lower than those of sugar beet pulp (5.0-10.0 g/g WRC and 10.5 mL/g SwC), citrus pulp (11.0 g/g WRC and 11.0 mL/g SwC), coconut fiber residue (5.3-7.1 g/g WRC and 20.0 mL/g SwC) and water-soluble (guar, citrus and apple pectin) gums (16.5-63.1 g/g WRC and 7.4-10.5 mL/g SwC) [7,11,30,32,33]. The ORC of CPHP is similar to those (0.9-1.30 g/g) of citrus peel, apple pomace, carrot pulp, pea hull, and wheat bran; but lower than those (4.3-5.2 g/g) of coconut residue fiber, sugar beet pulp, and yellow passion fruit rind byproducts [7,11,32].

3.5. Total Phenolics and Condensed Tannins of CPHP

The results of the TPC of CPHP and the amount of condensed tannins are summarized in Table 3.

Generally, the TPC of plant-derived (food) materials is determined from aqueous organic solvent-soluble phenolic fractions, obtained by a one-step extraction or a sequential extraction procedure using four kinds of organic solvents (acetone, chloroform, ethanol, and methanol). This is usually so done, because the TAOC of phenolic compounds in complex materials (such as foods) is usually attributed to the soluble fraction, consisting of low-molecular weight phenolics, which are partly absorbed in the gastrointestinal tract, despite their limited bioavailability, whereby they would contribute to the antioxidant status *in vivo*, whilst the high-molecular weight condensed tannins (proanthocyanidins) would remain in the intestinal tract, thereby limiting their potential bioactivity to this location [30]. However, this way of appraising the TPC and TAOC of complex food materials is currently criticized and often questioned [35] inasmuch none of these solvents would be capable of extracting all the antioxidant compounds from a complex food material, especially those associated with complex polysaccharides and proteins. As a consequence, a considerable amount of antioxidants remaining in the insoluble residues is usually ignored in most chemical and biological studies. And yet, these non-extracted antioxidants are (believed to be) released from the food matrix into the human gut by the action of digestive enzymes and intestinal microflora and may produce significant biological effects [35].

Table 3. Physicochemical and antioxidant properties of CPHP, CHPFR, and CKPFR

	CPHP	CHPFR	CKPFR
Physicochemical properties			
WRC (g/g)	6.49 ± 0.08a	3.89 ± 0.62b	ND
SwC (mL/g)	7.31 ± 1.14a	5.16 ± 0.11a	ND
ORC (g/g)	1.92 ± 0.05a	1.04 ± 0.06a	ND
Antioxidant properties			
Soluble phenolics (mg GAE/g)	68.93 ± 5.61a	26.71 ± 4.52b	22.61 ± 0.92b
Condensed tannins (mg/g)	52.16 ± 2.03a	92.43 ± 2.78 b	81.7 ± 1.15 b
DPPH (%)	85.42 ± 1.63a	67.51 ± 2.06b	62.9 ± 0.14b
DPPH [EC_{50} (g/g)]	24.96 ± 1.02a	43.26 ± 2.38b	55.14 ± 1.2b
ABTS (µmol TE/g)	51.87 ± 1.09a	10.52 ± 1.17b	2.48 ± 0.05c
FRAP (µmol TE/g)	129.5 ± 2.24a	83.46 ± 2.01b	61.27 ± 1.08c

Data are expressed as mean ± SD (n = 3). Values in the same line with different letters are significantly different at $P = .05$ according to the Bonferroni's test.
CPHP: "fresh" cacao pod husk product; CHPFR: fermented-and-roasted cocoa hull product; CKPFR: fermented-and-roasted cocoa kernel product
WRC: water-retention capacity; SwC: swelling capacity; ORC: oil-retention capacity
ND: not determined
DPPH: 2,2-Diphenyl-1-picrylhydrazyl; ABTS: 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; FRAP: ferric reducing antioxidant power
GAE: Gallic acid equivalent; TE: Trolox equivalent

Therefore, in this study, a four-step sequential extraction method [hot 80% aqueous ethanol (70-95°C), cold 60% aqueous acetone (23°C), lukewarm pure water (37°C), and hot acidified butanol (100°C)] has been designed for the evaluation of the TPC and TAOC of CPHP. The first three soluble fractions were combined and considered to be soluble phenolic compounds. Indeed, (60-70% aqueous) methanol and ethanol generally extract low-molecular weight phenolics, including oligomeric proanthocyanidins, whereas larger polymers (tannins) are better extracted with 60-70% aqueous acetone [36]. The last fraction, obtained with the Porter's reagent (acidified butanol), was assumed to be depolymerised condensed tannins [13], which constituted a quantitatively important organic-solvent-insoluble fraction of the dietary intake of antioxidants [35]. Thus, the TPC of CPHP was estimated at ~7.0% and appeared to be significantly higher ($P < 0.05$), compared with the TPC (~2.0-3.0%) of CHPFR and CKPFR Table 3. This indicated that cacao pod husks were richer in low-molecular weight phenolics and medium-molecular weight-proanthocyanidins than the hulls and kernels of fermented and roasted cocoa beans.

The organic solvent- and water-insoluble phenols which were extracted in the form of depolymerised condensed tannins, under more severe (acid) conditions using the Porter's reagent method, represented ~5.0% Table 3. Surprisingly, the amount of condensed tannins was significantly lower ($P < 0.05$) in CPHP than in CHPFR (~9.0%) or in CKPFR (~8.0%) Table 3. This may be explained by the fact that during fermentation of cocoa beans, oxidative polymerisation of phenolics occurs, which leads to the formation of insoluble protein-tannin complexes, the amounts of which are (substantially) augmented by insoluble materials generated by the formation of Maillard products during the subsequent roasting [29,31]. Soluble phenolics accounted for ~2.0% of CKPFR, indicating that the fermentation process of the cocoa beans used in this study (for comparison purposes) has correctly been conducted. The total amount of soluble polyphenols in the dried fat-free mass of fresh cocoa beans (Forastero var.) is, indeed, ~10.0–20.0%, equaling approximately 6.0% in air-dried cocoa beans with 54.0% fat and 6.0% moisture contents, and approximately 5.0% in fermented beans (10.0% and more being considered a sign of a bad fermentation) [28]. It should be underlined that three quantitatively different groups of polyphenols, viz. 37.0% catechins or flavan-3-ols, 4.0% anthocyanins and 58.0-60.0% proanthocyanidins have been identified in cocoa beans [6,28].

3.6. The total Antioxidant Capacity (TAOC) of CPHP

It should first be mentioned that a great number of methods have been designed for the analysis of the TAOC of complex plant-derived (food) materials, four of which, ORAC (oxygen radical absorbance capacity) [37], DPPH [25], FRAP [26], and ABTS [27] assays, are more commonly used. FRAP, ABTS, and DPPH are colorimetric methods (515-734 nm), which can be performed with the help of a colorimeter or a UV-Vis spectrophotometer, readily affordable by all laboratories, while ORAC requires a fluorimeter, which is not so common to many laboratories. Because the TAOC of a complex plant-derived material generally results from a mixture of various antioxidant compounds with different mechanisms of action, among which there may be synergistic (or antagonistic) interactions, there is not one analytical method that can provide unequivocal results [2,35]. Therefore, a better practice nowadays recommends the combination of (at least two) different methods for the *in vitro* determination of the TAOC of foodstuffs [2,30,38].

In this study, DPPH, ABTS, and FRAP assays were used to evaluate the TAOC of the three different cacao byproducts and the results obtained are presented in Table 3. The DPPH radical measures a sample's free radical scavenging capacity, which is based on the premise that a single electron transfer or a hydrogen donor (here soluble phenolic fractions from the cacao byproducts) is an antioxidant. This method is indeed widely used as a preliminary assay [2]. The results showed that more than 85.0% of the DPPH radical was inhibited by soluble phenolics from CPHP, whereas the inhibition percentage, caused by each of CHPFR and CKPFR was significantly lower, this being only in the range of 62.0-68.0%. Moreover, the EC_{50} (25.0 g/g) of soluble phenolics from CPHP was approximately two-fold lower than the EC_{50} (43.0-55.0 g/g) of CHPFR and CKPFR. These results

indicated that the TAOC, as judged by the DPPH assay, was greater in CPHP than in the other two cacao products.

The ABTS radical also measures a sample's free radical scavenging capacity. It is commonly referred to as a decolorization assay in which there is only a single electron transfer reaction. The TAOC of soluble phenolics from CPHP, as appraised by the ABTS assay, was ~52.0 µmol TE/g, and was significantly higher than that (2.0-11.0 µmol TE/g) of soluble phenolics from CHPFR and CKPFR, thereby confirming that CPHP was richer in free radical scavengers (antioxidant compounds) than the remainder.

It is should be mentioned that the (organic solvent and water-) insoluble residues, before treating them with the Porter's reagent, were found to induce ABTS radical decolorization, which indicated that they still contained free radical scavenging antioxidant compounds, probably chiefly high-molecular weight condensed tannins. However, the data obtained are not presented in the present study, because the extent to which these insoluble polyphenols would be released from food matrix by digestive enzymes and intestinal microflora into the human gut, whereby they would contribute to the antioxidant status *in vivo* remains unclear.

FRAP measures the ability of a sample to reduce metal cations and is also based on a single electron transfer reaction. The TAOC of soluble phenolics from CPHP, as evaluated by the FRAP assay, was ~130.0 µmol TE/g, and appeared to be significantly higher than the TAOC (61.0-83.0 µmol TE/g) of soluble phenolics from CHPFR and CKPFR. This indicated that CPHP was richer in metal-reducing antioxidant compounds than the other two products. In general, the values obtained here for soluble phenolics from the three Ivorian cacao byproducts (CPHP, CHPFR, and CKPFR) are higher than the one (72.32 µmol TE/ g) reported for a fiber-rich (hull-like) cocoa product [30]. Furthermore, strong correlation (R^2 = 96.8%) was found to exist between the TPC and TAOC determined by the FRAP assay. By contrast, the correlation between the two parameters, estimated by either DPPH or ABTS assay, was not found to be strong enough (R^2 = 78.9-82.3%).

Although the differing origin of cacao might contribute, to some extent, to discrepancies between our results and literature data, the higher TAOC observed for the three Ivorian cacao byproducts especially CPHP, could mainly be attributed to the extra extraction step using lukewarm (37°C) pure water. Analysis of the water-soluble fractions for glycosyl residues indeed revealed a quantitative presence of arabinose, galactose, rhamnose, and galacturonic acid, indicating that a pectic material was co-extracted with polyphenols. Pectic polysaccharides from another plant source (mangosteen) have recently been shown to be effective free radical (DPPH) scavengers [39]. The free radical scavenging activity and metal-reducing power of pectic polysaccharides could be explained by the presence of several protonated carboxyl (-COOH) groups (on polygalacturonic chains) and secondary alcohol (-OH) groups (on the galacturonic acid and neutral sugar residues of pectic chains), which render them potential hydrogen donors and contributors to the single electron transfer mechanism. It is therefore probable that the high TAOC of the soluble fractions from CPHP is the result of synergistic interactions among various compounds with antioxidant properties, including low-molecular weight phenolics, medium molecular weight proanthocyanindins and pectic-derived oligosaccharides and/or polysaccharides (mainly extracted during the pure-water step) from the cell wall matrix.

4. Conclusion

Study on unutilized cacao pod husks, from the Ivoirian cacao crops, for antioxidant-dietary fiber potential showed a high concentration of these health benefit components in this agrowaste. The amount of antioxidant-dietary fiber compounds appears to be higher in cacao pod husks than in fermented and roasted cocoa bean hulls and kernels. Therefore, cacao pod husks stand as a potential source of antioxidant-dietary fiber-rich food material, which may be used to compensate for their shortage or complete lack in refined modern diets currently associated to various free radical-induced disorders.

A list of Abbreviations Used

ABTS: 2,2'-Azinobis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; **ADF**: antioxidant-dietary fiber; **CHPFR**: cocoa hull product from fermented and roasted beans; **CKPFR**: cocoa kernel product from fermented and roasted beans; **CPHP**: cacao pod husk product from fresh pods; **DPPH**: 2,2-Diphenyl-1-picrylhydrazyl; **FRAP**: ferric reducing antioxidant power; **GAE**: gallic acid equivalent; **IDF**: insoluble dietary fiber; **NSP**: non-starchy polysaccharides; **ORAC**: oxygen radical absorbance capacity; **ORC**: oil-retention capacity; **ROS**: reactive oxygen species; **SDF**: soluble dietary fiber; **SwC**: swelling capacity; **TAOC**: total antioxidant capacity; (**T)DF**: (total) dietary fiber; **TE**: Trolox equivalent; **TPC**: total phenolic content; **TPTZ**: 2,4,6-Tri(2-pyridyl)-*s*-triazine; **WRC**: water-retention capacity;

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Competing Interests

"The authors declare that no competing interests exist."

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