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ORIGINAL ARTICLE

Characterization of Dutch-Cocoa produced using potash extract from cocoa pod husk as an alkalizing bioresource

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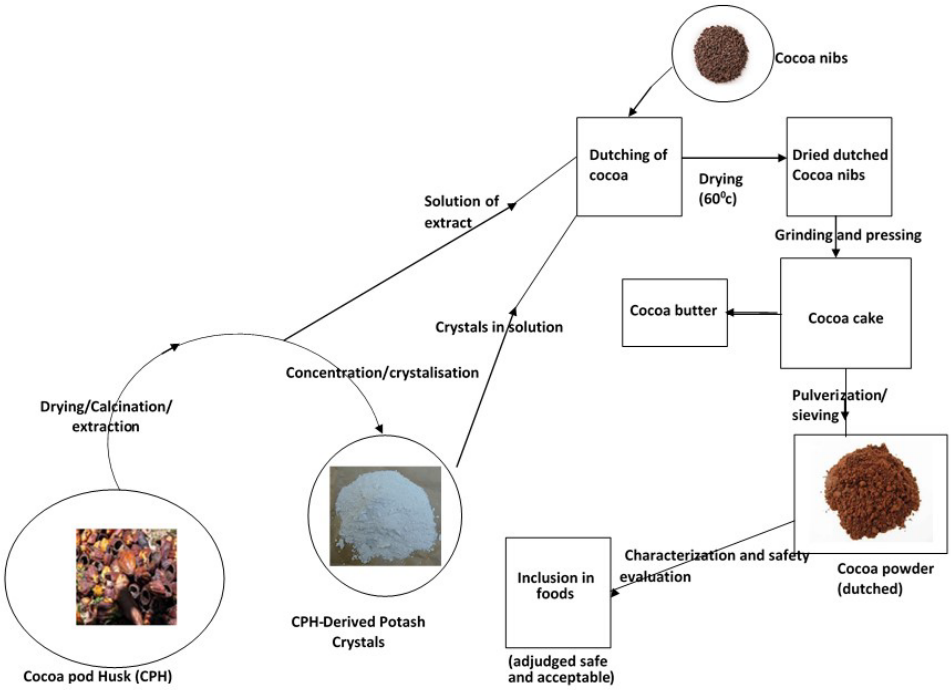
Abstract

Alkalizing agents in the processing of Dutch-Cocoa are often imported from developing countries. This occurs amidst humongous quantities of Cocoa Pod Husk (CPH) that are largely rotting away. This study therefore appraises the inherent alkalizing potentials of CPH, including its physicochemical and safety characteristics in the production of Dutch-Cocoa. CPH was calcined, potash extracted, characterized, and applied in formulation (1% to 5% conc.) in Dutch-production of Cocoa. Quality parameters of the resultant product were analyzed following AOAC procedures ($p \leq 0.05$). In addition, rats(n=30) were fed it over a 21-day duration while nutritional and safety indicators were monitored. Sensory properties were also evaluated. The results showed some predominant properties of CPH potash extract [Potassium 35.7%, pH 12.3, alkalinity 15.6 g/100 g CO_3] and Dutch-cocoa [protein (15.8% to 16.5%), colour (Hunter L,a,b) 36.9, 8.8, 11.7 light - dark red), dispersibility (1.5 to 2.3), wettability (143.7 s), sedimentation (20.7% to 49.3%)] which favourably compared with commercial variants. Apparent digestibility (AD%) was significant (Protein 86%, Fat 88%, Fiber 66% etc) ($p \leq .0.05$). Safety indices exhibited no deleterious effect and the product was adjudged acceptable. Dutch-cocoa produced using CPH-derived-potash as an alternate alkalizing bioresource is feasible, while simultaneously providing an environmentally friendly outlet for CPH

Keywords: Cocoa dutching; Alkalized cocoa; Cocoa-husk-based Alkalinizer; Food acid regulator; Cocoa potash; Dutch-cocoa; Food additive (alkalizing); Soluble cocoa; Chocolate beverage.

Graphical Abstract



Highlights

- Cocoa pod husk derived potash compares favourably with food grade commercial potash
- The potash extract equally enhanced the colour, flavour, and solubility of cocoa
- Toxicological tests underscored the safety of the potash extract in dutching cocoa

1 Introduction

Undoubtedly, cocoa (*Theobroma cacao* L) remains one of the most distributed and well-known agricultural products worldwide. Remarkably, over 75% of global output comes from Africa (International Cocoa Organization, 2020) and serves as raw material for most beverage, biscuits, and pastry industries (Miller et al., 2008). In response to consumers' demand, the cocoa is sometimes processed into Dutch cocoa – a variant with the darker colour, less astringent, bitter and acidic notes, but more soluble (Puchol-Miquel et al., 2021). Alkalizing agents such as potassium and sodium carbonates have been used in the processing of Dutch-cocoa (*T. cacao*) but imported by cocoa producing countries, as Nigeria. In many industries, they serve as authorized acidic regulator additives (Food and Agriculture Organization of the United Nations, 1981). Regrettably, scarce foreign exchange earnings are being expended on the importation of these alkalizing agents, despite the fact that cocoa pod husk's alkalizing potential is known but unexploited (Oduwole & Arueya, 1993; Kone et al., 2020).

Cocoa pod husk over the years has been generated by several cocoa producing countries as by-products. At the established ratio (dry cocoa beans: wet pods, 1:10) (Campos-Vegas et al., 2018) and according to the recent cocoa beans production (International Cocoa Organization, 2020), the magnitude of wet pods produced have increased [Cote d'Ivoire – 21, Ghana 8.0, Nigeria 2.5, Cameroon 2.8 million metric tonnes

etc]. Currently, these CPHs are largely discarded or just rot away at huge economic and environmental costs, affecting soil fertility and harboring the fungus causing black pod disease in cocoa plantations (Akanni & Ojeniyi, 2007; Osundahunsi et al., 2007). Proposals such as its application in fertilizer formulation (Djeke et al., 2011), hydrolase enzyme production (Yusof et al., 2016), source of bioactives (Campos-Vega et al., 2018) as well as soft soap and biogas production (Antwi et al., 2019) have not been translated into success stories. This situation may be associated with the cost and complexities related to the scale-up technology. Cocoa pod Husk as a source of potash for food and non-food applications is also well known. Indeed, it is best obtained after sun drying/open–hearth ashing followed by aqueous extraction and concentration/crystallization (Oduwole & Arueya, 1993). Though a relatively slow process, a significant part of the energy required to drive the operation comes from within. Meanwhile, the exploitation of the inherent rich potash content (Kone et al., 2020) in the production of Dutch-cocoa has not been evaluated. The burden of cocoa pod husk waste continues to rise and has become a serious challenge for waste management in many cocoa producing countries(Kouakou et al.,2018; Belwal et al., 2022). In the light of the foregoing, it is the objective of this study to extract potash from cocoa pod husk, purify, characterize, and apply the same in the processing of dutch cocoa with the imported commercial variant as the standard. Its digestibility and safety using the animal model will also be evaluated after the physicochemical, functional, and microbiological properties are established. This expectedly will reduce the importation of potash while saving hard foreign exchange and also providing a sustainable outlet for CPH to mitigate environmental pollution.

2 Materials and methods

2.1 Materials

Dried fermented cocoa beans and Cocoa Pod Husk (CPH) were obtained from the Cocoa Research Institute of Nigeria (CRIN), Ibadan, Nigeria. All reagents used during the present study were of analytical grade.

2.2 Extraction and characterization of potash from cocoa pod husk

The extraction of potash from CPH followed the method of Oduwole & Arueya (1993) with little modification. CPH was sun-dried and calcinated. Ash obtained was boiled for 20 minutes, cooled, and the filtrate concentrated and crystallized. The potash extract derived was analyzed to determine the following parameters namely: pH, potassium, sodium, colour, alkalinity, melting point, molarity, density, solubility (in water and alcohol) all according to standard methods (Association of Official Analytical Chemists, 2000). This evaluation was also done for its commercial variant.

2.3 Preparation of Dutch-cocoa powder

Dutch-cocoa powder was prepared according to the method described by Osundahunsi et al. (2007). Dried fermented cocoa beans were sorted, cleaned and extraneous materials were discarded. The dried cocoa beans were roasted at 126-130 °C to remove the shell and enhance the flavor. The cocoa nibs obtained were alkalized by soaking in the extracted potash(alkali) solution of different concentrations (1%, 2%, 3%, 4%, and 5%) at 60^0C for 2-3 hours and thereafter oven-dried at 100 °C to 120 °C for 2 hours. The dried samples were milled into a paste and the fat was extracted using a Lab. Manually operated hydraulic press (TMAX-RT-600D, UK). The cocoa cake was pulverized and sieved with 160 micron mesh size to obtain the powder. The alkalized cocoa powder was stored in ziploc bags.

2.4 Physicochemical and Compositional Analysis of Dutch-cocoa Powder

2.4.1 Determination of pH

A digital pH meter (Model 3520, Bibby scientific limited Dumow Essex,UK) on which the pH value for each sample was read directly was employed to determine the pH.

2.4.2 Colour determination

Colorimeter(Labscan XE) was used to measure the colour parameters of the Dutch-cocoa powder samples using Hunter L, a, b color standard and the average values were determined (Association of Official Analytical Chemists, 2005). The parameters recorded: L, a and b coordinates of the CIE scale. *L (lightness) axis – 0 is black, while 100 is white; *a (red-green) axis – positive values are red while negative values are green and 0 is neutral; *b (yellow-blue) axis – positive values are yellow, while negative values are blue and 0 is neutral. The chroma meter was calibrated with a standard white tile (L* = 86.4, a* = 0.3158, b* = 0.3236). The total color difference [DE= $(L^*- L)^2$ + $(a^* - a)^2$ + $(b^* - b)^2]^{1/2}$ was calculated from values for L*- (lightness), a*- (redness), b*- (yellowness).

2.4.3 Proximate compositional analysis of dutch-cocoa powder

The proximate composition of Dutch-cocoa powder was determined. These included moisture, ash, crude protein, crude fat, and total carbohydrates (Association of Official Analytical Chemists, 2000).

2.5 Functional properties of Dutch-cocoa powder

The functional properties of the Dutch-cocoa powder produced using the CPH derived potash extract were analyzed for Sedimentation (Selamat et al., 1998), Dispersibility (Lees, 1995), Wettability (Hansen,1980), Bulk Density (Owuamanam et al., 2010), Water and Oil Absorption Capacity (Owuamanam et al., 2010).

2.6 Microbiological analysis of Dutch-cocoa

Total mesophilic (i.e. total viable bacteria counts) and fungal counts (yeast and mold counts) were carried out on the sample of alkalised cocoa powder as described by Aidoo et al., 2010.

2.6.1 Serial dilution

Cocoa samples were prepared by mixing 90 ml of 0.1% peptone water. This was added to 10 g of the dried sample and homogenized in a stomacher for about 60s. Appropriate serial dilutions were prepared from the stock homogenate and used for the microbiological analyses.

2.6.2 Total Plate Count (TPC)

The total counts of the aerobic mesophilic bacteria were determined using the TPC method on Plate Count Agar (Oxoid Ltd., Basingstoke, Hampshire – England). The plates were incubated at 35^0C for 48 ± 2 h. The number of colonies was counted and recorded as colony forming units per gram of sample (cfu/g)

2.6.3 Yeast and molds determination

Yeast and mold populations in the samples were determined by plating on Malt Extract Agar (pH 5.4 Oxoid Ltd., Basingstoke, Hampshire - England) and incubating at 25 °C for 5 days. The number of colonies developed was counted and recorded as colony forming units per gram of sample (cfu/g).

2.7 Nutritional and Safety evaluations

2.7.1 Experimental design

Following approval of the research protocol by the Animal Care and Use Research Ethics Committee(ACUREC) of the University of Ibadan, male Wistar (albino) rats (n=25) of 5 to 6 weeks old with weights between 58 to 133 grams were obtained from the Department of Physiology, University of Ibadan. The animals were housed and acclimatized for 7 days. They were further subdivided into groups of five per replicate in five separate metabolic cages. Rats were housed individually in the rat metabolic cage in a well-ventilated room. In addition to their routine food and water, the experimental groups were fed 1ml of an aqueous cocoa suspension (2% conc.) daily for 21 days by oral gavage. The control animals were given the same volume of water over the same period using the same method (Abrokwah et al., 2009).

The entire feeding trials lasted for 28 days, during which time, weekly feed consumption and weight changes were monitored. On the 21st day of the feeding trial, total faeces and urine produced were collected from the rats for three consecutive days. At the end of the collection period, nitrogen in the test diets and dry faeces were determined by the method of the Association of Official Analytical Chemists (1995). The Nitrogen concentration in the dried faeces and test diets were used to compute the apparent digestibility (AD).

2.7.2 Haematological and Biochemical Parameters

Approximately 2ml of blood was drawn by cardiac puncture. One millilitre (1ml) of the blood was allowed to clot and centrifuged to obtain haemolysis-free serum and used for biochemical analyses. About 0.5 ml of blood was put into vials containing EDTA and used for haematological analyses (Benson et al.,1989). The haematological parameter evaluated included {Packed Cell Volume (PCV), Red Blood Count (RBC), White Blood Cell (WBC), Haemoglobin Concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), and Mean Corpuscular Haemoglobin (MCH) Platelet}, as well as biochemical indices [Serum protein, Triglyceride, total protein, Uric acid and Enzyme assays {Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP)}. were determined using a commercial kit (Randox Laboratories Ltd, U.K].

2.8 Sensory evaluation of Dutch cocoa powder

2.8.1 The Dutch-cocoa samples were first converted into a beverage:

Twelve grams (12g) of Dutch-cocoa powder and 15 g of sugar were weighed into a 400 ml glass beaker. Two hundred milliliters (200 ml) of tap water at 58 °C were added and stirred until a homogeneous suspension was achieved. About 20 ml of the suspension were put into a disposal cup and neatly covered.

2.8.2 Sensory Analysis

Each sample was coded and evaluated by thirty panelists using commercial alkalized cocoa powder based beverage as reference. The five coded experimental samples (Dutch-cocoa powder) and the reference cocoa powder were rated for colour, flavour/aroma, and overall acceptability. A hedonic scale of 1-9 where 1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely was applied

2.9 Statistical analysis

The results obtained were subjected to Analysis of Variance (ANOVA) to determine the variance ratio. Duncan's multiple range test (DMRT) was used to separate the means at 5% level of significance. SPSS (15.0) was used for data analysis.

3 Results and discussion

3.1 Physicochemical properties of the potash extract

The potassium (35.7%) and sodium (0.7%) compared favourably with the imported variant (Table 1) (Figure 1). The reduced sodium content coincidentally is in consonance with the essential requirements of World Health Organization(WHO) for a mean population sodium chloride intake of fewer than five grams per day by 2025 (Puchol-Miquel et al., 2021) The alkalinity (15.6 g/100 g CO_3) of the potash extract from CPH was lower than that of the imported sample (21.7 g/100 g CO_3) respectively. This is in accordance with the slightly lower pH value obtained for the potash extract from CPH. The melting point (854 °C) of potash extracted from CPH is favourably compared with the imported one (800 °C). [Potassium Carbonate (Analytical grade) (m.pt 899 °C) (Haynes, 2010)]. Impurities are known to lower the melting point of substances especially through distortion of the bonds holding the chemical structure of the compound in place. The impurity in the commercial sample may be a result of poor handling and storage at some point in the commercial chain (Rather et al., 2017). Further purification of the potash extract from CPH may yield purer samples, ostensibly with higher melting points.

Table 1. Physicochemical Properties* of Potash Extract.

Parameters	Potash extract from CPH	Imported potash
pH	12.3 ± 0.0	13.4 ± 0.0
Potassium (%)	35.7 ± 0.1	37.7 ± 0.0
Sodium (%)	0.7 ± 0.0	0.9 ± 0.0
Colour	Pale white	Pure white
Alkalinity (g/100g CO_3)	15.6 ± 0.9	21.7 ± 1.4
Melting point (°C)	854.0 ± 1.4	800.5 ± 0.7
Molarity (mol/dm^3)	0.5 ± 1.4	0.7 ± 1.4
Density (g/cm^3)	0.9 ± 0.0	1.31 ± 0.0
Solubility in water (at 37 °C)	90.5 ± 0.7	111.0 ± 1.4
Solubility in alcohol	Insoluble	Insoluble

*Determinations were measured in triplicates.

(a) (b)

Figure 1. Samples of Potash. (a) Commercial potash (b) CPH Potash extract.

3.2 Physicochemical properties of the Dutched cocoa

The pH of the experimental sample ranged from 6.3-7.3 (Table 2). These values are in agreement with the values reported by Gernard (1976) for alkalised cocoa powder which ranged from 6.5% to 7.20%. Nibs alkalization leads to very intense colours and increases in pH values. Alkalization has a salutary effect on the flavour of the final cocoa powder and produces colour varying from light brown, dark brown, dark red to charcoal black (Beckett, 1999). The colour of cocoa powder to a large extent is dependent on the level of fermentation and alkalization of the nibs during processing. The Dutch-cocoa powder samples produced from this research work falls between lightly and medium alkalized cocoa powder with the colour ranging from red-brown to brick-red. These colours are used to boost the flavour and appearance of various food products and are therefore important parameters for food manufacturers. Reports from various cocoa processing industries in Nigeria indicated that cocoa powders of varying pH are produced according to the customer's specifications (Adeyeye, 2016).

Table 2. Physicochemical properties of Colour and pH of Dutch-cocoa.

Samples	Colour				pH
	L	a*	b*	DE^+	
A	37.9 ± 0.01^{c}	9.3 ± 0.00^{a}	12.2 ± 0.01^{c}	50.7 ± 0.01^{bc}	6.3 ± 0.14^{e}
B	38.4 ± 0.29^{b}	8.5 ± 0.38^{a}	11.8 ± 0.11^{d}	50.0 ± 0.31^{b}	6.8 ± 0.14^{d}
C	36.9 ± 0.00^{d}	8.8 ± 0.01^{a}	11.7 ± 0.00^{d}	51.6 ± 0.00^{a}	7.0 ± 0.14^{c}
D	38.5 ± 0.35^{b}	7.4 ± 3.48^{a}	12.4 ± 0.01^{b}	50.3 ± 0.45^{bc}	7.0 ± 0.01^{b}
E	40.2 ± 0.00^{a}	9.6 ± 0.01^{a}	13.1 ± 0.00^{a}	47.8 ± 0.99^{d}	7.3 ± 0.01^{a}
F	38.1 ± 0.21^{b}	8.2 ± 0.30^{a}	11.7 ± 0.01^{d}	49.9 ± 0.30^{c}	6.5 ± 0.13^{d}
G	38.6 ± 0.30^{b}	8.5 ± 0.35^{a}	11.3 ± 0.09^{d}	50.3 ± 0.29^{b}	6.6 ± 0.13^{d}

Mean values not having the same superscript (a,b,c,d,e) down the columns are significantly different ($p < 0.05$). Sample A = Dutch-cocoa (1% potash). Sample B = Dutch-cocoa (2% potash). Sample C = Dutch-cocoa (3% potash). Sample D = Dutch-cocoa (4% potash). Sample E = Dutch-cocoa (5% potash). Sample F = commercial Dutch-cocoa (Stanmark). Sample G = commercial Dutch-cocoa (Tulip). DE^+ total color difference $[DE= (L^*- L)^2 + (a^* - a)^2 + (b^* - b)^2]^{1/2}$ was calculated from values for L*- (lightness), a*- (redness), b*- (yellowness).

3.3 Proximate composition of the Dutch-cocoa powder

The moisture content values (4.8-5.3%) for all the samples (Table 3) met the standard range considered safe for good keeping quality (Abiola & Tewe, 1991; ADM Cocoa, 2009) Alkalized/Dutch-cocoa powder was highly hygroscopic and both physical and chemical reaction may take place if the high relative humidity of the storage environment is allowed. The significant increase in the fat content of Dutch-cocoa powder across groups may be attributed to the use of a manually operated hydraulic press in the defatting process. The residual fat in the samples ranged from 16.3-18.2% exhibiting significant differences with samples A and B giving the highest and lowest values respectively. Most industrial users prefer cocoa powder with fat content (10% to 12%) achievable more with efficient industrial hydraulic presses (Melo et al., 2022) rather than Laboratory scale hydraulic press as used in this study. There was a sharp increase in the fiber content in sample E and may be due to possible interaction between the inherent polysaccharides, polyphenolics and Maillard products at some high temperatures during process operations (Redgwell et al., 2003). These values observed are greater than those reported (10.05% to 12.65%) by others (Nsor-Atindana et al., 2012; Ndife et al., 2013). Sample A exhibited the highest fiber content of 13.17 declining with an increase in potash concentration from sample B to D. However, there was a sharp increase in the fiber content in sample E. The protein content of the Dutch-cocoa powder ranged from 15.9% to 16.5%, *i.e.,* values lower than those of the controls (23.5% and 27.8%). This component rose steadily as the concentration of potash increased from 2% to 4%. The average ash content of the experimental samples was observed to be slightly lower than that of the control. It was highest in sample C, and lowest in sample A. The carbohydrate value was higher in sample D, and lowest in sample E. The experimental groups showed significantly different values compared to the controls which ranged from 39.3% to 39.7%.

Table 3. Proximate composition (%) of the Dutch-cocoa powder.

Samples	Moisture	Fat	Protein	Fiber	Ash	Carbohydrate
A	4.8 ± 0.07^{c}	18.2 ± 0.13^{a}	16.5 ± 0.07^{c}	13.2 ± 0.09^{a}	5.6 ± 0.0^{e}	41.8 ± 0.31^{c}
B	5.3 ± 0.07^{a}	16.3 ± 0.07^{c}	15.1 ± 0.03^{e}	13.0 ± 0.01^{a}	7.5 ± 0.0^{c}	42.9 ± 0.64^{b}
C	5.1 ± 0.07^{b}	17.2 ± 0.01^{b}	15.8 ± 0.08^{d}	11.6 ± 0.08^{b}	7.6 ± 0.0^{c}	42.7 ± 0.13^{b}
D	4.8 ± 0.07^{c}	16.3 ± 0.01^{c}	15.9 ± 0.01^{d}	10.7 ± 0.21^{c}	7.0 ± 0.0^{d}	45.4 ± 0.64^{a}
E	5.0 ± 0.00^{b}	17.5 ± 0.01^{b}	15.9 ± 0.00^{d}	13.1 ± 0.42^{a}	7.5 ± 0.2^{c}	41.0 ± 0.64^{c}
F	5.1 ± 0.05^{b}	12 ± 30.04^{d}	23.5 ± 0.07^{b}	11.0 ± 0.02^{b}	8.8 ± 0.0^{a}	39.3 ± 0.09^{e}
G	5.3 ± 0.05^{a}	10.7 ± 0.07^{e}	27.8 ± 0.03^{a}	8.7 ± 0.01^{d}	7.9 ± 0.0^{b}	39.7 ± 0.08^{d}

Mean values not having the same superscript (a,b,c,d,e,f) down the columns are significantly different ($p < 0.05$). Sample A = Dutch-cocoa (1% potash). Sample B = Dutch-cocoa (2% potash). Sample C = Dutch-cocoa (3% potash). Sample D = Dutch-cocoa (4% potash). Sample E = Dutch-cocoa (5% potash). Sample F = commercial Dutch-cocoa (Stanmark). Sample G= commercial Dutch-cocoa (Tulip).

3.4 Functional properties of the Dutched cocoa powder

The Dutch-cocoa exhibited good water (133.8-202.0g/100g) and Oil (128.9-163.0g/100g) absorption capacities (Table 4), properties not largely affected by the differences in concentration of the alkalizing agent. Notably, these data closely compare with values reported for the same properties of some cocoa powder samples in Cote d'Ivoire (Karim et al., 2020).The packed bulk density, a significant determinant in the choice of packaging material, ranged between 2.0 and 2.5g/ml depending among others on residual moisture content (Asiedu,1989).

Table 4. Functional properties of the Dutch-cocoa powder.

Samples	Water Absorption Capacity	Oil Absorption Capacity	Loose Bulk Density (g/ml)	Packed Bulk Density (g/ml)	Dispersibility	Wettability (s)	Sedimentation (%)
A	133.8 ± 0.64^{f}	128.9 ± 0.12^{d}	1.6 ± 0.35^{b}	2.0 ± 0.35^{b}	1.5 ± 0.01^{d}	169.7 ± 0.57^{c}	20.7 ± 1.15^{d}
B	202.0 ± 0.01^{a}	160.5 ± 0.74^{b}	1.6 ± 0.35^{b}	2.2 ± 0.35^{b}	2.0 ± 0.07^{b}	143.7 ± 0.57^{d}	49.3 ± 1.15^{a}
C	186.5 ± 0.71^{c}	156.2 ± 1.19^{c}	1.9 ± 0.35^{a}	2.5 ± 0.35^{a}	2.3 ± 0.71^{a}	242.7 ± 0.57^{b}	33.7 ± 3.21^{b}
D	156.8 ± 0.13^{e}	158.7 ± 1.15^{c}	1.7 ± 0.35^{b}	2.2 ± 0.35^{b}	1.7 ± 0.04^{c}	251.0 ± 1.00^{a}	28.3 ± 2.89^{c}
E	182.3 ± 0.64^{d}	163.0 ± 1.46^{a}	1.6 ± 0.35^{b}	2.2 ± 0.35^{b}	2.1 ± 0.11^{b}	137.0 ± 1.00^{e}	31.3 ± 2.31^{b}
F	200.0 ± 0.02^{a}	158.5 ± 0.74^{c}	1.6 ± 0.35^{b}	2.1 ± 0.35^{b}	2.0 ± 0.07^{b}	132.7 ± 0.57^{f}	47.3 ± 3.00^{a}
G	197.0 ± 0.01^{b}	160.5 ± 0.74^{b}	1.4 ± 0.35^{c}	2.2 ± 0.35^{b}	2.3 ± 0.07^{a}	123.2 ± 0.17^{g}	45.3 ± 2.10^{a}

Mean values not having the same superscript (a,b,c,d,e,f) across the columns are significantly different ($p < 0.05$). Mean values not having the same superscript (a,b,c,d,e,f,g) down the columns are significantly different ($p < 0.05$). Sample A = Dutch-cocoa (1% potash). Sample B = Dutch-cocoa (2% potash). Sample C = Dutch-cocoa (3% potash). Sample D = Dutch-cocoa (4% potash). Sample E = Dutch-cocoa (5% potash). Sample F = commercial Dutch-cocoa (Stanmark). Sample G = commercial Dutch-cocoa (Tulip).

There was marginal improvement in the dispersibilty of Dutch-cocoa samples as the concentration of alkali increased from 1% to 3%. Emulsifiers such as lecithin are sometimes added to cocoa powders to enhance their dispersibility (Compaore et al., 2011). The maximum and minimum sedimentations exhibited by sample B (49.3%) and sample A (20.7%) had an inverse relation with solubility. This phenomenon ostensibly may be related to the extent of degradation of ester linkages and cell wall residues by the Dutch-processed cocoa (Domínguez-Rodríguez et al., 2017). The wettability time for sample D (252s) was the highest and about twice that of sample E, *i.e.,* the lowest(137s). Wettability is an important product parameter in the production of chocolate beverages (Aidoo et al.,2010)

3.5 Microbiological analysis

The microbial populations of dutch cocoa samples are shown in Table 5. Evidently, there was no significant difference ($p<0.05$) in the values obtained for the TPC of the experimental samples and that of the two control. The microbial result showed that the number of microorganism present was negligible. The low moisture content observed limited the growth of microorganisms. Beckett (1999) and Mabbett (1998)

suggested that the quality criteria for cocoa mass include figures for a number of yeasts found per gram- maximum of 50 and for alkalised cocoa powder a normal maximum of 50 with a limit of 100. No coliform bacteria growths and salmonella were detected in any of the samples. This indicates that the samples are completely free of faecal contamination and pathogens. There was no significant difference in the values obtained for mold and yeast of the experimental samples and for the control.

Table 5. Microbiological Profile of the Dutch-cocoa powder.

Samples	Total plate count (cfu/g)	Mold (cfu/g)	Yeast (cfu/g)	E. Coli (cfu/g)	Salmonella (cfu/g)
A	11.5 ± 0.71^{a}	7.0 ± 1.41^{a}	5.0 ± 1.41^{a}	Nil	Nil
B	10.5 ± 0.71^{ab}	7.0 ± 0.00^{a}	3.5 ± 0.71^{d}	Nil	Nil
C	10.0 ± 0.00^{ab}	5.0 ± 1.41^{c}	4.0 ± 1.41^{c}	Nil	Nil
D	9.0 ± 1.41^{b}	4.0 ± 1.41^{d}	3.5 ± 0.71^{d}	Nil	Nil
E	10.0 ± 1.41^{ab}	7.0 ± 0.00^{a}	4.5 ± 0.71^{b}	Nil	Nil
F	9.6 ± 0.50^{ab}	6.0 ± 0.00^{b}	4.3 ± 0.51^{b}	Nil	Nil
G	8.7 ± 0.42^{ab}	7.1 ± 0.00^{a}	4.7 ± 0.60^{a}	Nil	Nil

Mean values not having the same superscript (a,b,c,d,e,f) down the columns are significantly different ($p < 0.05$). Sample A = Dutch-cocoa (1% potash). Sample B = Dutch-cocoa (2% potash). Sample C = Dutch-cocoa (3% potash). Sample D = Dutch-cocoa (4% potash). Sample E = Dutch-cocoa (5% potash). Sample F = commercial Dutch-cocoa (Stanmark). Sample G = commercial Dutch-cocoa (Tulip).

3.6 Animal studies

3.6.1. Body weight, food intake and food efficiency of rats fed Dutch-cocoa beverage

There was a significant difference ($p < 0.05$) in final weight, weight gain, food intake, and food efficiency among the experimental rat groups throughout the study period (Table 6). The rats that were fed with sample B (2% Potash in 100g of cocoa) had a loss in weight (8.6%) compared with other groups This was in sharp contrast with significant body weight gain (15.6%) of the control group that had no inclusion of Dutch-cocoa. This could possibly be attributed to not only the magnitude in variations in food intake but also to the presence of catechin and epicatechin - bioactives in cocoa linked to weight loss tendencies (Greenberg et al., 2016). Notably, rats that were fed with Sample E had the highest (12%) weight gain in the experimental group. The food intake generally ranged from 46.0 g to 54.3 g with rats that were fed with sample B, thus having the lowest food intake followed by sample D. There was also a significant difference in food efficiency of the experimental diet (-21.3 to 24,5%) in comparison to that of control (34.9%). Impact of Dutch-cocoa is closely related to the concentration of potash (Valverde García et al., 2020). Evidence has shown that it does enhance gut health through the polymerization of several smaller compounds which pass unabsorbed before reaching the large intestines where they are degraded by colonic bacteria releasing beneficial metabolites (Essenmacher, 2020).

Table 6. Food intake, body weight and food efficiency of rats fed with Dutch-cocoa beverage.

Groups	Initial body weight (g)	Final body weight (g)	Weight gain (g)	Food intake (g/week)	Food efficiency (%)
A	92.2 ± 0.21^{d}	96.9 ± 0.08^{e}	4.7 ± 0.13^{e}	54.3 ± 0.0^{a}	8.7 ± 0.23^{e}
B	113.7 ± 0.07^{a}	103.9 ± 0.07^{d}	-9.8 ± 0.0^{f}	46.0 ± 0.0^{d}	-21.3 ± 0.0^{f}
C	112.1 ± 0.14^{a}	123.5 ± 0.14^{a}	11.4 ± 0.0^{c}	53.3 ± 0.0^{a}	21.4 ± 0.01^{c}
D	106.5 ± 0.07^{b}	113.5 ± 0.14^{c}	7.1 ± 0.07^{d}	47.7 ± 0.0^{c}	14.8 ± 0.13^{d}
E	104.6 ± 0.21^{c}	117.3 ± 0.14^{b}	12.7 ± 0.07^{b}	51.7 ± 0.0^{b}	24.5 ± 0.11^{b}
F	108.3 ± 0.14^{b}	125.2 ± 0.28^{a}	16.9 ± 0.14^{a}	48.3 ± 0.0^{c}	34.9 ± 0.28^{a}

Mean values not followed by the same superscript (a,b,c,d,e,f) down the columns are significantly different ($p < 0.05$). Group A = Rats fed with Dutch-cocoa (1% potash). Group B = Rats fed with Dutch-cocoa (2% potash). Group C = Rats fed with Dutch-cocoa (3% potash). Group D = Rats fed with Dutch-cocoa (4% potash). Group E = Rats fed with Dutch-cocoa (5% potash). Group F = Rats fed with basal diet (control).

3.6.2 Nutrient intake, nutrient output and apparent digestibility of rats fed Dutch-cocoa beverage

The values observed for protein apparent digestibility (AD) of the experimental diet and control group ranged from 67% to 86% with sample E having the highest (Table 7). There were significant differences in the values obtained in the experimental diet group and that of the control. Sample D had the lowest protein AD (77%) followed by the control (67%). Generally, natural cocoa powder contains about 20% of crude protein, the digestibility of which is reported to range from 0 to 62% in rats (Eggun & Beames, 1986). Such a wide range of cocoa protein digestibility reflects the problems associated with feeding on cocoa diets. Firstly, there is a marked reduction in food intake concerning palatability challenges owing to the astringency of diets high in cocoa (James & Treloar, 1984). Similarly, the highest value for fat apparent digestibility was for rats fed with samples E and A (88%). The experimental groups were significantly higher in fat apparent digestibility (sample C 84%, B 83%, and D 80%) compared to the control in which 41% of the animals were not fed with the Dutch-cocoa beverage. Alkalization evidently enhances the hydrolysis of fats into fatty acids making them more digestible. It is the positional isomers of these fatty acids that determine the overall absorption and metabolism of the fats (Aoyama et al., 1996) The ash apparent digestibility followed the same trend with rats that were fed sample E (67%), thus having the highest value, followed by samples C (56%), A (52%), D (46%) and B(2%) when compared with the rats of the control group (18%). The rather low Ash AD observed for sample B is unclear but may not be unconnected with interactions during processing involving antinutritional factors making the inherent minerals unavailable for digestion. Rats fed with sample E (66%) had the highest value for fiber apparent digestibility followed by samples B (61%), A (52%), D (43%), and C (42%) when compared with the control group (6%). On the one hand, there was no significant difference in carbohydrate apparent digestibility of samples A (83%) and B (86%) and between samples C (87%) and D (85%); on the other hand, rats fed with sample E (91%) remained distinct, exhibiting the highest values.

Table 7. Nutrient intake (g), Nutrient output (g), and Apparent Digestibility - AD (%) of rats fed with Dutch-cocoa Beverage.

	A	B	C	D	E	F
Protein Intake	18.92 ± 0.21^{a}	15.44 ± 0.04^{d}	18.26 ± 0.06^{a}	16.34 ± 0.04^{c}	17.71 ± 0.01^{b}	8.86 ± 0.3^{e}
Protein Output	3.45 ± 0.04^{bc}	2.84 ± 0.01^{e}	3.86 ± 0.04^{a}	3.51 ± 0.01^{b}	2.30 ± 0.01^{f}	2.96 ± 0.01^{d}
AD	82 ± 0.00^{b}	83 ± 0.01^{b}	79 ± 0.01^{c}	77 ± 0.01^{c}	86 ± 0.1^{a}	67 ± 0.01^{d}
Fat Intake	12.52 ± 0.03^{a}	9.72 ± 0.03^{c}	11.74 ± 0.05^{b}	10.11 ± 0.01^{c}	11.60 ± 0.14^{b}	2.35 ± 0.14^{d}
Fat Output	1.49 ± 0.01^{c}	1.61 ± 0.14^{b}	1.74 ± 0.03^{b}	2.0 ± 0.00^{a}	1.39 ± 0.01^{d}	1.41 ± 0.01^{d}
AD	88 ± 0.00^{a}	83 ± 0.01^{b}	84 ± 0.14^{b}	80 ± 0.00^{c}	88 ± 0.00^{a}	41 ± 0.01^{d}
Ash Intake	7.04 ± 0.01^{c}	7.06 ± 0.01^{c}	8.04 ± 0.02^{a}	6.82 ± 0.03^{d}	7.69 ± 0.02^{b}	3.59 ± 0.02^{e}
Ash output	3.42 ± 0.01^{c}	7.0 ± 0.01^{a}	3.62 ± 0.01^{b}	3.61 ± 0.01^{b}	2.51 ± 0.01^{d}	0.18 ± 0.01^{e}
AD	52 ± 0.01^{c}	2.0 ± 0.14^{f}	56 ± 0.01^{b}	46 ± 0.01^{d}	68 ± 0.01^{a}	18 ± 0.01^{e}
Fiber Intake	12.08 ± 0.11^{a}	10.21 ± 0.00^{c}	11.10 ± 0.0^{b}	9.51 ± 0.01^{d}	11.53 ± 0.01^{ab}	4.50 ± 0.00^{e}
Fiber Output	6.01 ± 0.01^{b}	3.97 ± 0.01^{e}	6.41 ± 0.01^{a}	5.53 ± 0.01^{c}	4.01 ± 0.14^{de}	4.25 ± 0.00^{d}
AD	52 ± 0.01^{c}	61 ± 0.00^{b}	42 ± 0.00^{d}	43 ± 0.00^{d}	66 ± 0.01^{a}	6 ± 0.00^{e}
Carbohydrate Intake	50.61 ± 0.01^{a}	42.88 ± 0.01^{c}	50.09 ± 0.01^{a}	46.08 ± 0.01^{b}	47.18 ± 0.14^{b}	24.79 ± 0.00^{d}
Carbohydrate Output	8.81 ± 0.01^{a}	6.56 ± 0.01^{c}	6.52 ± 0.04^{c}	7.09 ± 0.01^{b}	4.63 ± 0.35^{d}	5.16 ± 0.00^{c}
AD	83 ± 0.00^{c}	86 ± 0.01^{b}	87 ± 0.01^{b}	85 ± 0.00^{b}	91 ± 0.14^{a}	79 ± 0.00^{d}

Mean values not accompanied by the same superscript (a,b,c,d,e) across the rows are significantly different ($p < 0.05$) (Post table data same as in Table 6 above).

3.6.3 Haematological parameters

There was no significant difference among the treatment groups except for the MCH parameters (Table 8) suggesting the non-toxicity of the potash extract. The MCH concentration (MCHC) obtained for sample E and control were the highest. This is also true for the neutrophils (excluding sample D) which had a lower value than others, but there were no significant differences compared with the other treatment and

control. This same pattern was evident for white blood cells, platelet, MCV, lymphocytes, monocyte, and eosinophils. The significant increase in white blood cells (responsible for fighting infections) of some of the experimental rats implied that the administration of alkalized cocoa could boost the immune system, since these cells are responsible for immune-protection and fighting foreign bodies. This is in accordance with an earlier observation by Addai (2009) that cocoa promotes superlative health by strengthening the immune system. Cocoa powder prevents many diseases particularly viral ailment (Addai, 2009). The highest value of 46.3% obtained as PCV for sample C favourably compared with other treatment groups including the control (PCV is the percentage volume of red cells in whole blood). The Haemoglobin concentrations also followed the same trend as the PCV with no significant difference ($p<0.05$) within the groups. The non-significant reductions in concentrations of haematological parameters in rats fed with alkalized cocoa powder is an indication that the oxygen carrying capacity of the animals' blood is largely unchanged. The major function of the red blood cells is to transport oxygenated haemoglobin, from the lungs to the tissues (Mayne, 1994). The mean values obtained for samples A, B, C, D, and E treated with 1%, 2%, 3%, 4%, and 5% of potash obtained from cocoa pod husk were not significantly different ($p<0.05$) from the control. The MCH for all treatment groups favourably compared with the control ($p < 0.05$). The MCV, MCH, and MCHC values together with those of RBC, PCV, and Hb (generally regarded as indicators of assessment of tendency toward anaemia) showed that the diet was neither toxic to red blood cells nor an impediment toward erythropoiesis (Bain et al., 2011). Polyphenols in cocoa are known to inhibit the activation of platelets (Murphy et al., 2003) and have been shown in samples A, C, and D (all samples with lower platelet values) when compared to the control, whereas the values from samples B and E were higher than control. A possible explanation may be differences in the relative bioavailability of cocoa polyphenols in the test diets.

3.6.4 Biochemical parameters

The total protein obtained among the groups favourably compared with the control and there was no significant difference as presented (Table 9). The non-significant difference in total plasma protein indicated that alkalized cocoa showed little or no effect on total plasma protein concentration. Since plasma proteins are produced in the liver, these results showed that the administration of cocoa alkalized with potash from cocoa pod husk may not have a significant effect on this aspect of liver function. Although uric acid levels of sample E associated with the experimental animals were a little bit higher than the control, the differences were not significant. This data once again suggests that alkalized cocoa has no significant effect on uric acid turnover. A high level of uric acid is known to cause gout, while also capable of acting as a potent antioxidant (De Oliveira & Burini, 2012). Indeed, uric acid and /or its salt derivative is an efficient scavenger of highly reactive and harmful oxygen species such as hydroxyl radicals, superoxide anion, singlet oxygen and oxygenated heme intermediates in high valence state (Chernecky & Barbara,2001; De Oliveira & Burini, 2012). Since low levels of blood triglycerides may assist in preventing diseases like stroke and hypertension, the reduced blood triglycerides observed, will enhance a healthy living. Stearic acid, a saturated fatty acid abundant in cocoa is easily converted to oleic acid – a monounsaturated fatty acid, which has no deleterious effect on health. Stearic acid has also been reported to facilitate the reduction of plasma cholesterol by limiting its absorption while enchancing the execretion of endogenous cholesterol (Schneider et al., 2000).

The Alkaline phosphatase (ALP) levels in the experimental groups were generally not significantly different from the control. Experimental results established for Alanine aminotransferase (ALT) among the treatment groups were virtually the same. A similar pattern was evident for Aspartate aminotransferase (AST). Over the period of administration of the alkalized cocoa powder to test animals, no significant changes were observed in the levels of ALT, Aspartate aminotransferase (AST), and ALP. These enzymes are used to identify hepatocellular diseases, and evaluate their progression as well as bone and cardiac diseases (Osakabe et al., 2001). Injury or disease affecting these vital organs results in the release of these enzymes into the bloodstream, thus elevating their levels. Evidently, with these serum enzyme levels, administration of cocoa alkalized with CPH potash had no detrimental effect on the functioning of the organs involved.

Table 8. Heamatological parameters (%) of rats fed with Dutch-cocoa.

Groups	PCV	Hb	RBC	WBC	Platelet	MCV	MCHC	MCH	Neutrophils	Lymphocytes	MO	EO
A	43.0 ± 0.00^{a}	14.2 ± 0.2^{a}	7.2 ± 0.02^{a}	5.5 ± 0.77^{a}	328.0 ± 382.85^{a}	60.03 ± 0.06^{a}	33.0 ± 0.45^{abc}	19.8 ± 0.31^{a}	33.7 ± 6.56^{a}	61.3 ± 7.37^{a}	2.0 ± 1.00^{a}	2.3 ± 0.58^{ab}
B	42.7 ± 1.15^{a}	14.1 ± 0.36^{a}	7.1 ± 0.87^{a}	5.0 ± 0.40^{a}	820.0 ± 275.70^{a}	59.8 ± 0.95^{a}	33.1 ± 0.21^{abc}	19.8 ± 0.35^{a}	31.0 ± 7.94^{a}	65.3 ± 9.04^{a}	1.7 ± 0.58^{a}	2.0 ± 1.00^{ab}
C	46.3 ± 1.53^{a}	15.2 ± 0.47^{a}	7.6 ± 0.51^{a}	6.5 ± 2.44^{a}	282.0 ± 275.70^{a}	61.3 ± 2.21^{a}	32.9 ± 0.53^{bc}	20.1 ± 1.00^{a}	35.3 ± 7.09^{a}	61.0 ± 7.55^{a}	2.7 ± 0.58^{a}	1.0 ± 1.00^{b}
D	43.3 ± 7.23^{a}	14.2 ± 2.25^{a}	7.0 ± 1.43^{a}	6.4 ± 0.84^{a}	387.0 ± 44.27^{a}	62.4 ± 3.36^{a}	35.8 ± 0.44^{c}	20.5 ± 1.37^{a}	24.0 ± 3.46^{a}	70.7 ± 4.16^{a}	2.0 ± 1.00^{a}	3.0 ± 1.00^{a}
E	43.7 ± 4.73^{a}	15.5 ± 2.63^{a}	7.3 ± 0.87^{a}	5.0 ± 0.66^{a}	753.3 ± 113.72^{a}	59.7 ± 1.52^{a}	35.4 ± 2.11^{a}	21.1 ± 1.22^{a}	28.7 ± 8.02^{a}	67.3 ± 7.51^{a}	2.3 ± 1.15^{a}	1.7 ± 0.58^{ab}
F	42.3 ± 5.51^{a}	15.0 ± 2.76^{a}	7.2 ± 0.91^{a}	6.0 ± 2.48^{a}	52.0 ± 337.84^{a}	59.0 ± 0.66^{a}	35.2 ± 1.96^{bc}	20.8 ± 1.17^{a}	31.7 ± 1.53^{a}	68.0 ± 3.00^{a}	2.3 ± 1.15^{a}	1.3 ± 1.53^{ab}

Key: PCV = Packed Cell Volume; Hb = Hemoglobin. RBC = Red Bood Cell; WBC = White Blood Cell; MCV = Mean Corpuscular Volume; MCHC = Mean Corpuscular Hemoglobin Concentration; MCH = Mean Corpuscular Hemoglobin; MO =Monocytes; EO = Eosinophils. Mean values not accompanied by the same superscript (a,b,c,d,e,f) down the columns are significantly different ($p < 0.05$). Group A = Rats fed with Dutch-cocoa (1% potash). Group B = Rats fed with Dutch-cocoa (2% potash). Group C = Rats fed with Dutch-cocoa (3% potash). Group D = Rats fed with Dutch-cocoa (4% potash). Group E = Rats fed with Dutch-cocoa (5% potash). Group F = Rats fed with basal diet (control).

Table 9. Biochemical parameters of rats fed Dutch-cocoa.

Groups	Protein	AST	ALT	ALP	Triglycerides	Uric acid
A	7.00 ± 0.44^{a}	43.0 ± 3.61^{a}	32.0 ± 1.73^{a}	114.0 ± 5.19^{a}	1.00 ± 0.60^{a}	1.53 ± 0.91^{a}
B	7.77 ± 0.44^{a}	46.0 ± 2.00^{a}	33.0 ± 1.00^{a}	114.3 ± 6.51^{a}	0.87 ± 0.29^{a}	1.03 ± 1.18^{a}
C	6.57 ± 1.21^{a}	41.3 ± 4.04^{a}	30.0 ± 4.36^{a}	118.7 ± 12.1^{a}	1.03 ± 0.59^{a}	1.10 ± 1.21^{a}
D	6.70 ± 0.62^{a}	42.0 ± 3.61^{a}	32.0 ± 2.65^{a}	113.0 ± 5.29^{a}	0.87 ± 0.21^{a}	1.10 ± 0.75^{a}
E	6.80 ± 0.46^{a}	41.6 ± 5.69^{a}	30.7 ± 4.51^{a}	120.0 ± 9.54^{a}	0.77 ± 0.26^{a}	2.43 ± 0.35^{a}
F	7.87 ± 0.40^{a}	40.7 ± 2.89^{a}	29.3 ± 3.21^{a}	113.3 ± 10.0^{a}	0.37 ± 0.26^{a}	1.90 ± 1.32^{a}

KEY: AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline Phosphatase. Mean values not accompanied by the the same superscript (a,) across the columns are significantly different ($p < 0.05$). Group A = Rats fed with Dutch-cocoa (1% potash). Group B = Rats fed with Dutch-cocoa (2% potash). Group C = Rats fed with Dutch-cocoa (3% potash). Group D = Rats fed with Dutch-cocoa (4% potash). Group E= Rats fed with Dutch-cocoa (5% potash). Group F= Rats fed with basal diet (control).

3.6.5 Mortality rate

It is noteworthy that none of the test animals died either naturally or due to the feed ingestion.

3.7 Sensory evaluation

Each of the samples A to G was coded as 201, 301, 322, 502, 403, 207, and 504, respectively (Table 10). The results for taste and colour showed a similar score trend. Sample A was least preferred for taste (5.6) and colour (6.7) attributes, while sample G had the highest score (6.4) for taste and sample F (7.3) for colour. The significant difference observed in colour is due to the level of alkalization. Across the alkalised product samples, there was no significant difference in terms of flavour and overall acceptability. The overall acceptability scores recorded for all CPH test samples indicated that sample E was the most preferred although not significantly different from others including the two commercial variants.

Table 10. Sensory evaluation of the Dutch-cocoa.

Samples	Taste	Colour	Flavour	Overall Acceptability
A	5.6 ± 0.63^{b}	6.7 ± 0.70^{b}	7.5 ± 0.64^{a}	7.3 ± 0.59^{a}
B	5.7 ± 0.62^{b}	6.7 ± 0.70^{b}	7.5 ± 0.64^{a}	7.3 ± 0.59^{a}
C	6.0 ± 0.53^{ab}	7.0 ± 0.53^{ab}	7.5 ± 0.64^{a}	7.5 ± 0.52^{a}
D	6.1 ± 0.52^{ab}	7.1 ± 0.70^{ab}	7.5 ± 0.52^{a}	7.3 ± 0.62^{a}
E	6.1 ± 0.59^{a}	7.1 ± 0.64^{ab}	7.4 ± 0.49^{a}	7.5 ± 0.52^{a}
F	6.4 ± 0.99^{a}	7.3 ± 0.62^{a}	7.7 ± 0.49^{a}	7.7 ± 0.49^{a}
G	6.4 ± 0.99a	7.1 ± 0.64^{ab}	7.7 ± 0.49^{a}	7.7 ± 0.49^{a}

Mean values not having the same superscript (a,b) down the columns are significantly different ($p < 0.05$). Sample A = Dutch-cocoa (1% potash). Sample B = Dutch-cocoa (2% potash). Sample C= Dutch-cocoa (3% potash). Sample D = Dutch-cocoa (4% potash). Sample E = Dutch-cocoa (5% potash). Sample F = commercial Dutch-cocoa (Stanmark). Sample G = commercial Dutch-cocoa (Tulip)

4 Conclusion

The results of a physicochemical and functional evaluation of the Dutch-cocoa powder favourably compared with their commercial variants. In terms of digestibility and degree of wholesomeness, the experimental samples favourably compared with the control. The results showed that the use of potash extract from cocoa pod husk as an alkalising agent may be a possible substitute for the imported ones in the Dutching process. Application of the potash extract in the production and processing of cocoa powder should be encouraged.

References

Abiola, S. S., & Tewe, O. O. (1991). Chemical evaluation of cocoa by-products. *Tropical-Agriculture*, *64*, 335-336.

Abrokwah, F. K., Asamoah, K. A., & Esubonteng, P. K. A. (2009). Effects of the intake of natural cocoa powder on some biochemical and haematological indices in the rat. *Ghana Medical Journal*, *43*(4), 164-168. PMid:21326996.

Addai, F. (2009). *Intake of pure cocoa, source of healthy living.* Retrieved in 2020, August 10, from http://www.modernghana.com/news/217980/1

Adeyeye, E. I. (2016). Proximate, Mineral and Antinutrient Compositions of Natural Cocoa Cake, Cocoa Liquor and Alkalized Cocoa Powders Sourced in Nigeria. *Journal of Advanced Pharmaceutical Science and Technology*, *1*(3), 12-28. http://dx.doi.org/10.14302/issn.2328-0182.japst-15-855

ADM Cocoa. (2009). *De Zaan® Cocoa manual.* Switzerland.

Aidoo, H., Sakyi-Dawson, E., Tano-Debrah, K., & Saalia, F. K. (2010). Development and characterization of dehydrated peanut-cowpea milk powder for use as dairy milk substitute in chocolate manufacture. *Food Research International*, *43*(1), 79-85. http://dx.doi.org/10.1016/j.foodres.2009.08.018

Akanni, D. I., & Ojeniyi, S. O. (2007). Effect of different levels of poultry manure on soil physical properties, nutrient status, growth and yield of tomato (*Lycopersicon esculentum*). *Research Journal of Agronomy*, *1*, 1-4.

Antwi, E., Engler, N., Nelles, A., & Schüch, A. (2019). Anaerobic digestion and the effect of hydrothermal pretreatment on the biogas yield of cocoa pod. *Waste Management*, *88*, 131-140. PMid:31079625. http://dx.doi.org/10.1016/j.wasman.2019.03.034

Aoyama, T., Fukui, K., Taniguchi, K., Nagaoka, S., Yamaoto, T., & Hashimoto, Y. (1996). Absorption and metabolism of lipids in rats depend on fatty acid isomeric position. *The Journal of Nutrition*, *126*(1), 225-231. PMid:8558305. http://dx.doi.org/10.1093/jn/126.1.225

Asiedu, J. J. (1989). *Processing tropical crops: A technological approach* (pp. 24-42). London: Macmillan Education Ltd.

Association of Official Analytical Chemists – AOAC. (1995). *Official method of analysis of the Association of Official Analytical Chemists* (16th ed.). Washington.

Association of Official Analytical Chemists – AOAC. (2000). *Official methods of analysis of the Association of Official Analytical Chemists* (16th ed., pp. 834-841). Virginia.

Association of Official Analytical Chemists – AOAC. (2005). *Official method of analysis of the Association of Official Analytical Chemists* (18th ed.). Washington.

Bain, B., Bates, I., Laffan, M., & Lewis, S. (2011). Laboratory methods used in the investigation of hemolytic anemia. In M. Houston (Ed.), *Dacie and Lewis practical hematology* (pp. 229-243). London: Elsevier.

Beckett, S. T. (1999). *Industrial chocolate manufacture and use*. Glasgow: Blackie Academic & Professional.

Belwal, T., Cravotto, C., Ramola, S., Thakur, M., Chemat, F., & Cravotto, G. (2022). Bioactive compounds from cocoa husk: Extraction, Analysis and applications in food production chain. *Foods*, *11*(6), 798. http://dx.doi.org/10.3390/foods11060798

Benson, H J., Gunstream, S. E., Talaro, A,, & Talaro, K. P. (1989). *Anatomy and physiology laboratory textbook.* Dubuque: IOWA.

Campos-Vega, R., Nieto-Figueroa, K. H., & Oomah, B. (2018). Cocoa (*Theobroma cocoa* L) pod husk: Renewable source of bioactive compounds. *Trends in Food Science & Technology*, *81*, 172-184. http://dx.doi.org/10.1016/j.tifs.2018.09.022

Chernecky, C. C., & Barbara, J. B. (2001). *Laboratory test and diagonostic procedures* (pp. 147-156). Philadephia: WB Saunders.

Compaore, R. W., Nikiema, A. P., Bassole, N. H., Savadogo, A., Hounhouigan, D., Mouecoucou, J., & Traore, A. S. (2011). Nutritional properties of enriched local complementary flours. *Advance Journal of Food Science and Technology*, *3*(1), 31-39.

De Oliveira, E. P., & Burini, R. C. (2012). High plasma uric acid concentration: Causes and consequences. *Diabetology and Syndrome*, *4*(12), 1-7. http://dx.doi.org/10.1186/1758-5996-4-12.

Djeke, M. D., Angui, P. K. T., & Kouadio, J. Y. (2011). Decomposition of ground cocoa shells in ferrallitic soils in the Oume zone, West Central Coted'Ivoire: Effects on soil characteristics. *Biotechnologie, Agronomie, Société et Environnement*, *15*(1), 109-117.

Domínguez-Rodríguez, G., Marina, M. L., & Plaza, M. (2017). Strategies for the extraction and analysis of non-extractable polyphenols from plants. *Journal of Chromatography. A*, *1514*, 1-15. PMid:28778531. http://dx.doi.org/10.1016/j.chroma.2017.07.066

Eggun, B. O., & Beames, R. M. (1986). Use of Laboratory animals as models for studies of nutrition of domestic animals. In E. J. Ruitenberg & P. W. J. Peters (Eds.), *Laboratory animals* (pp. 265-290). Amsterdam: Elsevier Science Publishers.

Essenmacher, L. A. (2020). *Gut health benefit of natural and alkalized processed cocoa (Theobroma cacao) with and without Inulin* (Doctoral Dissertation). Virginia Tech, Virginia.

Food and Agriculture Organization of the United Nations – FAO. Codex Alimentarius. (1981). *Codex standard for cocoa powders (cocoas) and dry mixtures of cocoa and sugars. Codex stan. 105* (pp. 1-4). Rome: FAO.

Gernard, I. D. (1976). *Introductory food chemistry* (pp. 245-250). Oxford: Wiley.

Greenberg, J. A., O'Donnell, R., Shurpin, M., & Kordunova, D. (2016). Epicatechin, procyanidins, cocoa, and appetite: A randomized controlled trial. *The American Journal of Clinical Nutrition*, *104*(3), 613-619. PMid:27510533. http://dx.doi.org/10.3945/ajcn.115.129783

Hansen, P. L. (1980). Production of agglomerated fat filled milk powder. *International Journal of Dairy Technology*, *33*(1), 19-23. http://dx.doi.org/10.1111/j.1471-0307.1980.tb01459.x

Haynes, W. M. (2010). *CRC handbook of chemistry and physics* (91st ed., pp. 4-82). Boca Raton: CRC Press.

International Cocoa Organization – ICCO. (2020). *Quarterly bulletin of cocoa statistics - 2019/20 Cocoa year* (Vol. XLVI, No. 4). Côte d'Ivoire.

James, K. A. C., & Treloar, B. P. (1984). The comparative effects of orange roughy *(Hoplostethus at/anticus)* and snapper *(Chrysophrys auratus)* in the diets of growing rats. *New Zealand Journal of Science*. In press.

Karim, K. J. C., Saki, S. J., Sea, T. B., Yoboue, G. A., Kouassi, L., & Soro, Y. R. (2020). Functional properties of cocoa bean powder of the Mercedes and Theobroma cocoa varieties from the Lôh-djiboua and Indenie-djuablin regions (Cote d'Ivoire). *GSC Biological and Pharmaceutical Sciences*, *12*(1), 62-71. http://dx.doi.org/10.30574/gscbps.2020.12.1.0195

Kone, K., Akueson, K., & Norval, G. (2020). On the production of potassium carbonate from cocoa pod husks. *Recycling*, *5*(3), 1-6. http://dx.doi.org/10.3390/recycling5030023

Kouakou, E. K., Amed, C., Kouakou, F. K., Konan, C. M., Mouroufu, A. K., Kpan, E. S., Bouafou, K. G., & Kati Coulibaly, S. (2018). Cocoa pod in the ivorian plantations: Green gold neglected and bulky. *Nutrition & Food Science*, *7*(2), 1-3.

Lees, R. (1995). *Food analysis: Analytical and quality cocoa method for the food manufacturer and buyer* (3rd ed., 160 p.). London: Leonard Hill's Book.

Mabbett, T. (1998). Mycotoxin hazards: more accurate quantification in the field. *Feed International*, *25*, 39-42.

Mayne, P. (1994).*Clinical chemistry in diagnosis and treatment* (6th ed., pp. 281-323). New York: Oxford University Press.

Melo, L. J., Orjuela, A., & Guerrero, E. (2022). Improvement of industrial processes in Columbia. Case study: Cocoa pressing process in a chocolate factory. *Chemical Engineering Research & Design*, *183*, 307-317. http://dx.doi.org/10.1016/j.cherd.2022.05.013

Miller, K. B., Hurst, W. J., Payne, M. J., Stuart, D. A., Apgar, J., Sweigart, D. S., & Ou, B. (2008). Impact of alkalization on the antioxidant and flavanol content of commercial cocoa powders. *Journal of Agricultural and Food Chemistry*, *56*(18), 8527-8533. PMid:18710243. http://dx.doi.org/10.1021/jf801670p

Murphy, K. J., Chronopoulos, A. K., Singh, I., Francis, M. A., Moriarty, H., Pike, M. J., Turner, A. H., Mann, N. J., & Sinclair, A. J. (2003). Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *The American Journal of Clinical Nutrition*, *77*(6), 1466-1473. PMid:12791625. http://dx.doi.org/10.1093/ajcn/77.6.1466

Ndife, J., Bolaji, P., Atoyebi, D., & Umezuruike, C. (2013). Production and quality evaluation of cocoa products (plain cocoa powder and chocolate). *American Journal of Food and Nutrition*, *3*(1), 31-38.

Nsor-Atindana, J., Zhong, F., Mothibe, K. J., Bangoura, M. L., & Lagnika, C. (2012). Quantification of total polyphenolic content and antimicrobial activity of cocoa (*Theobroma cacao* L.) bean shells. *Pakistan Journal of Nutrition*, *11*(7), 574579.

Oduwole, O. O., & Arueya, G. L. (1993). Economic feasibility for potash prouduction from cocoa-pod husk. In *11th International Cocoa Research Conference*, Yamoussoukro, Cóte d'Ivoire.

Osakabe, N., Baba, S., Yasuda, A., Iwamoto, T., Kamiyama, M., Takizawa, T., Itakura, H., & Kondo, K. (2001). Daily Cocoa intake reduces the susceptibility of low-density lipoprotein to oxidation as demostrated in healthy human volunteers. *Free Radical Research*, *34*(1), 99-118. PMid:11235000. http://dx.doi.org/10.1080/10715760100300091

Osundahunsi, O. F., Bolade, M. K., & Akinbinu, A. A. (2007). Effect of cocoa ash as an alkalizing agent on cocoa products. *Journal of Applied Sciences*, *7*(12), 1674-1678. http://dx.doi.org/10.3923/jas.2007.1674.1678

Owuamanam, C. I., Ihediohanma, N. C., & Nwaneke, E. C. (2010). Sorption Isothermal, particle size, chemical and physical properties of cocoyam corn flours. *Science Pub*, *2*, 8.

Puchol-Miquel, M., Palomares, C., Fernández-Segovia, I., Barat, J. M., & Perez-Esteve, E. (2021). Effect of the type and degree of alkalization of cocoa powder on the physico-chemical and sensory properties of sponge cakes. *Lebensmittel-Wissenschaft + Technologie*, *152*, 112241. http://dx.doi.org/10.1016/j.lwt.2021.112241

Rather, I. A., Koh, W. Y., Paek, W. K., & Lim, J. (2017). Sources of chemical contaminants in food and their health implications. *Frontiers in Pharmacology*, *8*, 830-838. PMid:29204118. http://dx.doi.org/10.3389/fphar.2017.00830

Redgwell, J. R., Trovato, V., & Curti, D. (2003). Cocoa bean carbohydrates: roasting-induced changes and polymer interactions. *Food Chemistry*, *80*(4), 511-516. http://dx.doi.org/10.1016/S0308-8146(02)00320-5

Schneider, C. L., Cowles, R. L., Stuefer-Powell, C. L., & Carr, T. P. (2000). Dietary stearic acid reduces cholesterol absortion and increases endogenous cholesterol in hamsters fed cereal-based diets. *The Journal of Nutrition*, *130*(5), 1232-1238. PMid:10801924. http://dx.doi.org/10.1093/jn/130.5.1232

Selamat, J., Hussin, N., Zain, A. M., & Man, Y. B. C. (1998). Effects of alkalized cocoa powder and soylecithin on physical characteristics of chocolate beverage powders. *Journal of Food Processing and Preservation*, *22*(3), 241-254. http://dx.doi.org/10.1111/j.1745-4549.1998.tb00348.x

Valverde García, D., Pérez Esteve, É., & Barat Baviera, J. M. (2020). Changes in cocoa properties induced by the alkalization process: A review. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 2200-2221. http://dx.doi.org/10.1111/1541-4337.12581

Yusof, F., Khanahmadi, S., Amid, A., & Mahmod, S. S. (2016). Cocoa Pod Husk, a new Source of hydrolase enzyme for preparation of cross-linked enzyme aggregate. *SpringerPlus*, *5*, 57. PMid:26904389. http://dx.doi.org/10.1186/s40064-015-1621-3

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