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Evaluation of Nutritional, Phytochemicals, Microbiological and Sensory Properties of Cookies Enriched with Cocoa Bean Shells

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ABSTRACT: Cocoa bean shells (CBS) are wastes generated by cocoa processing industries despite its high nutritional properties. Production of cookies enriched with CBS in order to evaluate the effects of its addition on the processed snack food was studied. Cookies were prepared with multipurpose flour, butter, sugar, baking powder, salt, milk and varying quantities of CBS (0, 2, 4, 6, 8, 10g). The preparation of the cookies was done according to the normal method of cookies production. Nutritional, minerals, phytochemicals, microbiological and sensory evaluations were performed on the processed snack food to determine the contribution of the CBS in enhancement of these properties. Results of chemicals, minerals, phytochemicals and sensory analysis indicates increase in protein, fat, fiber and ash whereas moisture and carbohydrate content decreases as the quantity of the enricher increases. All the parameters determined for minerals and the phytochemicals also increase with increased addition of the CBS. The result of sensory analysis showed that all the samples were rated highly but for the sample with highest addition of CBS which had darker colour and aftertaste due to the deep brown colour of CBS. Microbial examination of the cookies indicated that total bacteria counts were low for all the sample ($1.7x10^2$-$3.1x10^3$) and no enteric bacteria was detected. The study shows that the cookies enriched with cocoa bean present a profile of mould in different percentages. There is however, an allowance of $1.0x10^4$ cfu/g for yeasts and moulds for baked products to cookies belong.

KEYWORDS: by-products, Cocoa beans shell, Cookies, Enrichment, Nutrition.

INTRODUCTION

The fruit of the pods of cacao tree normally called "*Theobroma cacao L*." is the dried and fermented fatty seed called cocoa beans. It consists of an outer shell surrounding two cotyledons and a small germ. It is an important and economic crop in developing countries like Cote D'Ivoire, Ghana, Nigeria and Cameroon. The production of cocoa beans takes place mainly in the tropical areas, to the tune of more than 4.7 million tons per year all over the world. About 76.3%, 17.4%, and 6.3%, were estimated to be produced in Africa, America, and Asia and Oceania, respectively, during the harvest season of 2018/2019 (González et. al, 2018). Fermented and dried cocoa beans are the principal raw material for chocolate production. Cocoa beans processing involves two stages namely preprocessing usually carried out in the farmers field and processing which are done in the industries in chocolate production.

In chocolate production, only 10% of the total cocoa fruit weight is used while the remaining whooping 90% is discarded as waste or by-products (Battegazzore et. al. 2014 and Chandrasekaran, 2012). One of these by-products is the external coatings that cover the cocoa beans, also known as cocoa bean shells (CBS), which are generated during the cocoa bean roasting process. The estimated generation of this material is about 700 thousand tons (Fowler, 2009; ICCO, May 2018). However, in the cocoa processing industries, the cocoa beans shells and germs represent the main by-products since they are exported together with the beans unlike other by-products of cocoa such as the cocoa pod husk, cocoa mucilage that are generated during the preprocessing stage and are normally discarded at the processing sites.

The increasing demand for cocoa beans as major raw material for chocolate production has led to the accumulation of this by-product, representing a serious disposal problem that could be aggravated by legal restrictions (Battegazzore et. al.2014). In order to understand the enormity of the cocoa bean shell (CBS) being generated as waste product, it was reported by Afrane and Ntiamoah, (2011) that the production of one kg of chocolate would produce an output of 98 g of CBS. The disposal of CBS could constitute important economic implication through added costs through extra weight during transport, cost of disposal, and its impact on the environment.

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However, the use of agricultural by-products through bioconversion of food processing residues into valuable products has begun to receive increasing attention so as to reduce cost of waste management and serious environmental pollution. As such assessment of cocoa bean shell has received increasing attention in several studies in order to find new applications for this by-product. Many researchers propose the use of these by-products in applications such as food ingredients, or other value-added applications among which are feedstuff for livestocks, industrial use as biofuel, and as an absorbent among other things (Olga et al.; 2020). Cocoa shells are just one of the examples of by-products with high-value bioactive components and interesting nutritional value that have been discarded. Cocoa bean shell consist of compounds that add functional properties like theobromine, phenolics and dietary fiber to the material to which it is added (Jahurul et al., 2013; Yusof, Khanahmadi, Amid, & Mahmod, 2016).

Thus, a further exploration of these products as additives in foods, food fortification or supplements of high nutritional value has gained increasing interest. This is as a result of their nutritional characteristics and for the fact that their recovery can be economically attractive (Murthy & Naidu, 2012). However, CBS present a much lower percentage of fats compared to cocoa beans, which is substituted by a much higher quantity of fibers (Esteban et al., 1994).

According to Chronopoulos et. al, (2011), it is possible to use milled cocoa shells, without any modifications, as well as to alkalize cocoa shells, and then use them as a food additive. Also, Nsor-Atindana et. al, (2012); Vitola and Ciprovica (2016) noted that relatively high values of dietary fiber together with phenolic compounds, with the implication that CBS as a by-product is fascinating to the food industries of confectionery and bakery products as a natural ingredient in the preparation of low calorie dietetic and fiber-rich food products. Okiyama et al (2017) reported that CBS contains a reasonable fat content with a very interesting lipid profile, similar to that of cocoa butter product. It also has an appealing brown chocolate color and chocolate flavor which enables its application as a unique natural colorant and a flavoring agent.

Cookies are a ready-to-eat confectionary food product which is either hard or crisp usually available in different forms, shape, and flavor. They are a popular foodstuff that has a wide range of consumption by people of all age group due to their varied taste, long shelf-life, relatively low cost and convenience. According to Awan et al., (1999) it's nutrient composition like vitamins, mineral elements, and especially dietary fiber is not adequate to make biscuits a balanced diet foods with substantial energy having wholesome and nutritious quality. There is increased demand for healthy, natural and functional foods by the consumers which is not unconnected with improved awareness and health consciousness of the modern-day consumers. Biscuits have ability to serve as vehicles for important nutrients to improve its nutritional value (Akubor, 2003; Hooda and Jood, 2005). Thus, together with the major ingredients used in cookies production which includes flour, fat, sugar, salt, milk and water, the addition of a natural functional ingredient that can boost the nutritive value of cookies and confer functional properties on it is a novel value-addition to cookies production. The nutritive value of cookies can be improved by fortification with a wide variety crude fiber-rich natural ingredient. (Masoodi and Bashir, 2012 and Tyagi et al., 2007 reported the fortification and supplementation of biscuits with crude fiber-rich plant products, such as psyllium, chicory, cereals bran, and germ. Also, Forzana and Mohajan, (2015), Ullah et al., (2016) reported that biscuits are been enriched with mushrooms and alfalfa seed flour the addition of which results in the increased nutritional and sensory properties of the products. The inclusion of cocoa bean shell to enrich corn snack products was done by Jozinovi´c et al. (2017) and they concluded that it can be successfully employed as nutritional fortification agent.

The present work is carried out to study the effects cocoa beans shell enrichment of biscuits on nutritional, functional, minerals, microbial and organoleptic properties of biscuits.

MATERIALS AND METHODS

Materials

Cocoa beans whose CBS was used for the experiment were obtained from a cocoa farmer's store at Ile Oluji, in Okeigbo Local government area of Ondo state, Nigeria. The CBS was recovered after roasting, cracking and winnowing of the beans. The CBS used were carefully sorted to make sure they are free of embryo and adhered broken nibs. Whole-wheat flour, margarine (fat), sugar, milk, baking powder and salt were purchased from Bodija market in Ibadan, Oyo State. All the chemicals used in this study are of analytical grade.

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METHODS

Preparation of Cocoa Bean Shells Paste (CBSP)

Preparation of cocoa beans shells was done following different stages of post-harvest primary processing stages of fermentation, drying and storage. Sorting of the beans to remove any extraneous materials like stones, flat beans, moldy beans, placenta and clustered beans was done.

Roasting of the beans was done at a temperature of 120ºC for 25 minutes which lead to the production of desirable chocolate flavours and colour. Breaking was done immediately with the aid of manual breaking machine after which cooling of the beans was done using cocoa beans cooler for 5 minutes for efficient breaking. Also, to ensure that no off-flavours are picked up from the environment, it was winnowed to remove the shells from the cocoa nibs. The recovered shells were rid of the embryo, adhered cocoa nibs slatty beans as may appear after breaking of the beans. Mechanical milling of the cleaned shells was done with the aid of beater blade mills so as to facilitate easy blending with the remaining ingredients to give good flavour and mouthfeel using Panasonic super mixer grinder, Model No. MX-AC, 220-230V~50-60Hz, 550W. Panasonic Appliances India Co., Made in India.

Table 1. Cookies Formulation

Samples	Wheat Flour/g	Butter/g	Sugar/g	Milk/g	Baking Powder/g	Salt/g	CBS/g
YK	100	50	60	45	2	1	0
KU	100	50	60	45	2	1	2
NK	100	50	60	45	2	1	4
RT	100	50	60	45	2	1	6
EN	100	50	60	45	2	1	8
AY	100	50	60	45	2	1	10

Preparation of Cookies

The cookies were prepared in the food processing laboratory of Cocoa Research Institute of Nigeria. Biscuits were made according to the method described by Omoba and Omogbemile (2013) with some modifications. The ingredients and the formulations used includes wheat multi-purpose flour (100g), sugar(60g), butter (50g), baking powder (2g), sodium chloride (1g) and full cream milk (45g). Cookies preparation was carried out using wheat multipurpose flour together with the remaining ingredients and zero quantity of CBS as control sample while other samples contain 2, 4, 6, 8 and 10g of cocoa bean shells. Each formulation was prepared separately. The sugar used in the cookie's preparation was grinded to fine powder using Panasonic super mixer grinder before use. All the ingredients were carefully weighed out for each formulation. Sugar and butter were creamed with the aid of the mixer while the flour, baking powder and salt were mixed manually before the addition of the creamed butter and full cream liquid milk. The two were mixed gently to form a dough. The dough was rolled and flattened on a platform with the aid of a roller pin to make thin sheets before cutting using different shapes cutters. The cut out shaped dough was placed in baking tray lined with paper foil and butter paper to prevent it from burning. It was baked in a Crown Star Oven (Trident (HK) Limited) China with in-built timer and tray regulator, 220V-240V, 50 Hz, 1500W which was pre-heated before it was baking at 180ºC for 15 minutes. After baking, the cookies were allowed to cool for 1hr after which it was packed in airtight plastic container for 24 hours before being subjected to chemical, phytochemical, microbial, mineral analysis and sensory assessment.

Proximate Analysis

Proximate composition of the enriched cookies (moisture, crude protein, crude fiber, crude fat, ash and carbohydrate) was determined in duplicate according to the standard methods of analysis described by the Association of Official Analytical Chemist (A.O.A.C., 18TH Edition, 2010).

Determination of Moisture Content

About 5 gram of each sample was weighed W_1 into a known weight of dried petri-dishes. The weight of the petri-dishes and the sample were taken and recorded as W_2 using electronic weighing balance. In reducing the moisture samples, petri-dishes containing the samples were placed into a pre-set oven at 105C for 6 h. the petri-dishes containing the weighed samples were then weighed as

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W_3. This was done until the weight of the samples were constant. Moisture content was determined by difference and expressed as a percentage.

$$\%\ Moisture = \frac{W_2 - W_3}{W_1} \times \frac{100}{1}$$

Determination of Crude Protein

The macro-khedjal method as described by the standard method of the association of the official analytical chemist (AOAC) (2005) was used to determine the crude protein. This consists of three techniques of analysis namely Digestion, Distillation and Titration.

A. Digestion: 0.5 gram of each sample was weighed carefully into the Kjeldahl digestion tubes to ensure that all sample materials got to the bottom of the tubes. To this were added 1 Kjeldahl catalyst tablet and 10 ml of Concentrated H_2SO_4. These were set in the appropriate hole of the Digestion Block Heaters in a fume cupboard. The digestion was left on for 4 h, after which a clear colorless solution was left in the tube. The digest was cooled and carefully transferred into 100 ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

B. Distillation: The digest solution was transferred into a clean khedjal distillation apparatus. The digestion flask was rinsed twice with 2 ml of deionized water to ensure that no residue was left. The distillation was done with Markham Distillation Apparatus which allows volatile substances such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steamed out for about ten minutes. The steam generator is then removed from the heat source to all the developing vacuum to remove condensed water. The steam generator is then placed on the heat source (i.e., heating mantle) and each component of the apparatus was fixed up appropriately.

C. Determination: 5 ml portion of the digest above was pipetted into the body of the apparatus via the small funnel aperture. To this was added 5 ml of 40% (W/V) NaOH through the same opening with the 5 ml pipette. The mixture was steam-distilled for 2 minutes into a 50 ml conical flask containing 10 ml of 2% Boric Acid plus mixed indicator solution placed at the receiving tip of the condenser. The Boric Acid plus indicator solution changes colour from red to green showing that all the ammonia liberated have been trapped.

D. Titration: The green color solution obtained was then titrated against 0.01 N HCL contained in a 50 ml Burette. At the end point or equivalent point, the green color turns to wine color which indicates that all the Nitrogen trapped as Ammonium Borate $[(NH_4)_2BO_3]$ have been removed as Ammonium chloride (NH_4CL).

The percentage nitrogen in this analysis was calculated using the formula:

$$\%\ Nitrogen = Titre\ value \times Atomic\ mass\ of\ Nitrogen \times Normality\ of\ HCL\ used \times 4$$

Or $\%\ Nitrogen = Titre\ value \times NormalityMolarity\ of\ HCL\ used \times Atomic\ mass\ of\ N \times Volume\ of\ flask\ containing\ the\ digest \times \frac{100}{1}$

$Weight\ of\ sample\ digested\ in\ milligram \times Vol.of\ digest\ for\ steam\ distillation.$

The crude protein content is determined by multiplying percentage Nitrogen by a constant factor of 6.25.

i.e $\%CP = \%N \times 6.25$

Determination of Crude Fibre: Approximately 2 gram of defatted sample was transferred into a 250 ml Erlenmeyer flask and 0.5 gram of asbestos added. About 200 ml of boiling 1.25% sulphuric acid (H2SO4) was added and immediately transferred unto a heating mantle. A cold finger condenser was attached to it and the sample boiled for 30 minutes. The content of the flask was filtered with linen cloth placed in a funnel. The residue was washed with boiling water until the washing was no longer acidic (determined using blue litmus paper). The washed sample with asbestos and then washed back into the flask (previously washed) with 200 ml of boiling 1.25% of sodium hydroxide (NaOH) solution. The flask was reconnected to the condenser and allowed to boil for 30minute. The mixture was filtered through a linen cloth and the residue washed with about 300 ml of boiling water and with 25 ml of alcohol. The residue was transferred into a previously dried and weighed crucible and dried at 100°C for 1hr in an oven, cooled in a desiccator at room temperature and then ignited at 600°C for 30 minutes after which it was cooled and reweighed. The loss in weight after ignition was determined and expressed as percentage crude fibre.

$$\%\ Crude\ Fibre = \frac{Weight\ of\ crude\ fibre}{Weight\ of\ sample\ used} \times \frac{100}{1}$$

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Determination of Crude Fat Content: The soxhlet apparatus was used in the extraction. Normal (n) hexane BP 60-68C was used as solvent for the extraction. A 500 ml round bottom flask was filled to ¾ of its volume with the solvent. 10 ml of milk sample was weighed into a 250 ml Conical Flask. 1ml of ammonium hydroxide was added, stoppered and well shaken.10 ml of ethyl alcohol was added and flask shaken again followed by the addition of 25 ml diethyl ether, shaken and 25 ml of petroleum ether with vigorous shaken. The flask was allowed to stand for 30 minutes after vigorous shaken. At the end of 30 minute, the ethereal layer was siphoned into a clean dry previously weighed sohxlet flask. The sohxlet containing the ethereal layer with the fat was put on the heating mantle of the sohxlet apparatus to distill off the solvent. The sohxlet flask + fat was removed, dried in an oven to a constant weight at 80^0C. The flask +fat was cooled in a desiccator and weighed. Percentage fat was obtained by the formula:

$$\%\ Crude\ Fat\ = \frac{Weight\ of\ extracted\ fat}{Weight\ of\ sample} \times 100$$

Determination of Ash Content: About 5 grams of the sample were weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550 ºC and left for about 4 h. About this time, it had turned to white ash. The crucible and its content were cooled to about 100 ºC in air, then room temperature in a desiccator and weighed. This was done in duplicate. The percentage ash was calculated from the formula below:

$$Ash\ content\ = \frac{weight\ of\ ash}{original\ weight\ of\ sample} \times \frac{100}{1}$$

Determination of Carbohydrate Content: The carbohydrate content was estimated by difference as shown below:

$$Carbohydrate(\%) = 100 - \%(protein + crude\ fat + ash + crude\ fibre + moisture)$$

Phytochemical Analysis

Total Phenolic Content: The concentration of phenolic in the sample powder was determined using spectrophotometric method (Singleton *et al*,1999). Ethanol solution of the extract in the concentration of 1mg/ml was used in the analysis. The reaction of the mixture was prepared by mixing 1ml of ethanolic solution of extract,2.5ml of 10% folin ciocalteu's reagent dissolved in water and 2ml of 7.5% NaHCo3 is added, blank was prepared. The sample were thereafter left for 30min.The absorbance was determined using spectrophotometer at 610nm, the sample were prepared in triplicate for each analysis. The same procedure was repeated for the standard solution of galic acid and the calibraton line was constructed based on the measured absorbance, the concentration of phenolic was read (mg/g) from the calibration line; then the content of phenolic in extract was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Total Flavonoid Content: Total flavonoid content were measured according to a colorimetric assay (Rajeev *et. al*., 2012). A 1-ml aliquot of standard solutions of catechin at different concentrations, or appropriately diluted samples was added to a 10ml volumetric flask containing 4ml double distilled water. At zero time, 0.3ml of 10% ALCl3 was added. After 6min,2ml of 1M NaOH was added to the mixture; the solution was immediately diluted to volume (10ml) with double-distilled water and thoroughly mixed. Absorbance was read at 510nm with a spectrophotometer.

Determination Of Tannin: Tannin was quantitatively determined as reported in the manual of food quality control (AOAC,1984). 0.5g of the sample was weighed into a conical and mixed with 10ml of 80% ethanol. This was shaken and allowed to stand for 1hr about 1ml of the extract was pipette into another test tube. This was followed by the addition of 5ml of distilled water. Two drops of Fecl in 0.1M Hcl was added. It was shaken to mix properly and about 4 drops of potassium ferrocyanide was also added, the absorbance of the portion of the mixture was read at 620nm using spectrophotometer

Caffeine Extraction Procedure: An aliquot (5 mL) of the sample was drawn with a 10 mL pipette and placed into a 125 mL separating funnel followed by the addition of distilled water (10 mL), then 20% aqueous Na_2CO_3 solution (1 mL) and analytical grade CCl_4 (20 mL). The caffeine was extracted by inverting the funnel at least three times, venting the funnel after each inversion. The non-aqueous CCl_4 layer was removed to a clean 50 mL volumetric flask. Another 20 mL portion of CCl_4 was added to the aqueous solution in the separating funnel and the extraction procedure was repeated twice more and the CCl_4 solvent layers combined. This volume was made up to 50 mL with the solvent. This procedure was repeated for all the samples. The absorbance of the resulting solutions was then measured on UV/Vis spectrophotometer at 270 nm (Mumin *et. al*., 2006).

Formular: =Conc(µg/ml) *Vol/ml*DF/Wt of Sample

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Analysis of Mineral Elements Composition

A. Dry Ashing Method: 2g of dry sample was weighed into a porcelain crucible and ashed at 550°C for 3hrs. The crucibles were allowed to cool and the ash was dissolved with 100ml of 3N HCl. It was stored in a plastic bottle with a plastic cap and taken to AAS for readings

B. Spectrophotometric Determination: In the Atomic Absorption Spectrophotometer (AAS), corresponding lamps were placed for corresponding mineral or heavy metal and the wavelength was set for the specific metal to be determined. AAS siphoning hose was put into the digested sample after running the standards for the metal determined. The concentration of the metal in the solution was displayed on the screen of the AAS machine. (AAS Used**:** Bulk Scientific; Model: 2010)

Microbiological Analysis. Total bacterial, Yeast, and molds count were determined according to the procedure described by Harrigan and McCance (1978).

Sensory Evaluation: Sensory analysis was carried out using 9-point Hedonic scale as described by (Iwe, 2002). The samples were evaluated by ten (10) semi-trained panelists who are frequent cookies eaters. Attributes examined includes aroma, colour, taste, aroma, crunchiness, texture, aftertaste and overall acceptability with 1 representing the least score (dislike extremely) and 9 the highest score (like extremely).

Statistical Analysis: The experimental data obtained from sensory evaluations were subjected to analysis of variance using statistical software SAS (2009,) while the means were separated using Carl Fisher's LSD at the degree of confidence of 0.05.

RESULTS AND DISCUSSION

Proximate Composition of the Enriched Cookies

The chemical constituents of the enriched cookies are as presented in table 1 below. Sample YK is the control sample that was not enriched with cocoa beans shell (i.e 0% CBS), while samples KU, NK, RT, EN and AY contains 2, 4, 6, 8 and 10% CBS flour weight basis respectively. The control sample YK contains the highest moisture while the least moisture was recorded for sample AY that contains the highest quantity of CBS in the range of 5.67 -3.33 %. Also, carbohydrate content follows the same trend as the moisture with the range of 67.23 – 49.30% for the control and the treated samples. This effect of CBS on moisture of content of the cookies is supported by the findings of Choi et al., (2019), where they reported improved color and moisture content of pork sausages enriched with CBS. However, reverse is the case for crude protein, crude fat, crude fibre and ash content for the control sample and the enriched samples. CBS enriched cookies recorded the lowest figures for the control sample while the values increased as the percentage inclusion increases. For protein, the values range from 26.17 to 11.18%, for fat, it is 9.33 to 5.33%, for fibre, it is 14.63 to 7.38 while that of ash range from 6.33 to 3.22%. Incorporation of by-products of fruits processing industries for enhancement of nutritional content of extruded products was reported by Suman and Rajinder (2015). They reported increase of 6.0 to 13.28 % in dietary fiber and 10.20 to 11.80% in protein when apple pomace powder was added to extruded product at three different levels. Also, Ebru and Mehmet, (2009) in their study at four different levels, enrich noodles with apricot kernel which is a by-product of apricot processing plants, the results indicated that the samples enriched with apricot kernel powder improved chemically for all addition levels as compared to control sample. The outcome of these studies has supported the findings of cocoa beans shell enrichment of cookies which has resulted in nutritional balance of carbohydrate rich cookies with more protein, fat, fiber and ash as compared to control sample. It also shows lesser moisture content than the control samples which will translate to a longer shelf life of the enriched product.

Table 2. Results of chemical evaluation of enriched Cookies

Sample	Moisture Content (%)	Crude Protein (%)	CrudeFat (%)	CrudeFibre (%)	Ash (%)	Carbohydrate (%)
YK	5.67±0.29^{a}	11.18±0.04^{f}	5.33±0.58^{c}	7.38±0.42^{e}	3.22±0.10^{c}	67.23±0.54^{a}
KU	4.83±0.12^{a}	13.11±0.01^{e}	5.67±0.58^{c}	11.44±0.70^{d}	4.45±0.19^{b}	62.01±0.10^{b}
NK	4.07±0.40^{b}	13.94±0.07^{d}	7.00±1.00^{b}	12.49±0.06^{c}	5.33±0.00^{ab}	56.40±0.85^{c}
RT	3.73±0.64^{b}	14.43±0.98^{c}	7.67±0.58^{b}	13.54±0.12^{b}	5.33±0.88^{ab}	54.63±0.48^{d}
EN	3.37±0.40^{b}	15.32±0.10^{b}	8.00±0.00^{b}	14.59±0.46^{a}	5.67±0.58^{a}	53.40±0.80^{e}
AY	3.33±0.58^{b}	26.17±0.01^{a}	9.33±0.58^{a}	14.63±0.11^{a}	6.33±0.69^{a}	49.30±0.95^{f}

Means with different superscripts within the same column are significantly different ($p < 0.05$)

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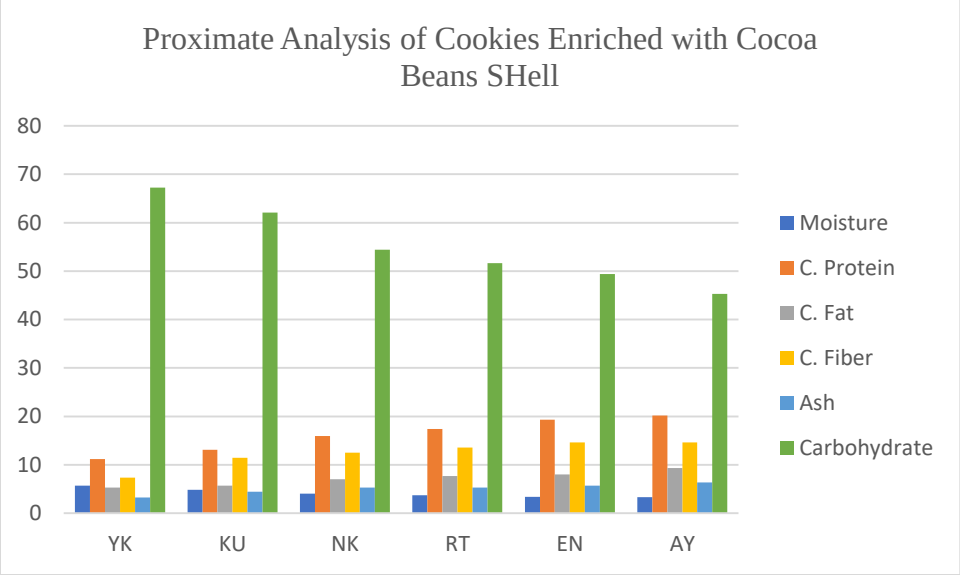
YK- 0% CBS, KU- 10% CBS, NK- 20% CBS, RT- 30% CBS, EN- 40% CBS AND AY-50% CBS.



Mineral Content (Mg/100g) Of CBS Enriched Cookies

Minerals are elements that are essential for the maintenance of human health by helping the body to perform its biochemical functions. They are needed for the proper composition of body fluids including blood for the proper composition of tissues, bone, teeth, muscles and nerves. Minerals also support healthy cardiovascular system. Minerals maintain their nutritional value through the cooking process and are not degraded by heat unlike vitamins. Thus, incorporation of minerals in cookies through the addition of cocoa beans shell can help to prevent nutritional imbalance. The results of minerals analysis of CBS enriched cookies are as indicated in table 2. There is increase in all the minerals assessed from the control sample YK in the range of 11.73 to 13.33 mg/g for iron, 371.13 to 589.18mg/g for potassium, 429.16 to 851.57mg/g for sodium, copper which is a micro mineral is the least abundant recorded the range of 0.12 to 0.20mg/g while phosphorus which is the most abundant mineral has a range of 573.33 to 822.67mg/g. this result indicated improvement in the mineral composition from control

.

Table 3. Minerals Composition of Cookies Enriched with Cocoa Bean Shell

Sample	Iron mg/g	Potassium mg/g	Sodium mg/g	Cupper mg/g	Phosphorus mg/g
YK	11.73±0.10^{d}	371.13±5.59^{f}	429.16±5.75^{f}	0.12±0.00^{f}	573.33±9.24^{e}
KU	12.08±0.11^{c}	393.13±0.00^{e}	565.39±0.00^{e}	0.13±0.00^{e}	790.67±10.07^{d}
NK	12.92±0.10^{b}	501.59±5.75^{d}	598.59±5.75^{d}	0.16±0.00^{d}	738.67±2.31^{c}
RT	12.98±0.10^{b}	540.73±3.23^{c}	635.12±0.00^{c}	0.18±0.00^{c}	812.1±10.58^{bc}
EN	13.04±0.00^{ab}	568.68±8.59^{b}	794.49±9.96^{b}	0.19±0.00^{b}	822.67±16.17^{b}
AY	13.33±0.37^{a}	589.18±5.59^{a}	851.57±11.50^{a}	0.20±0.00^{a}	904±27.71^{a}

Means with different superscripts within the same column are significantly different ($p < 0.05$)
YK=0g CBS, KU= 2g CBS, NK=4g CBS, RT=6g CBS, EN=8g CBS and AY= 10g CBS.

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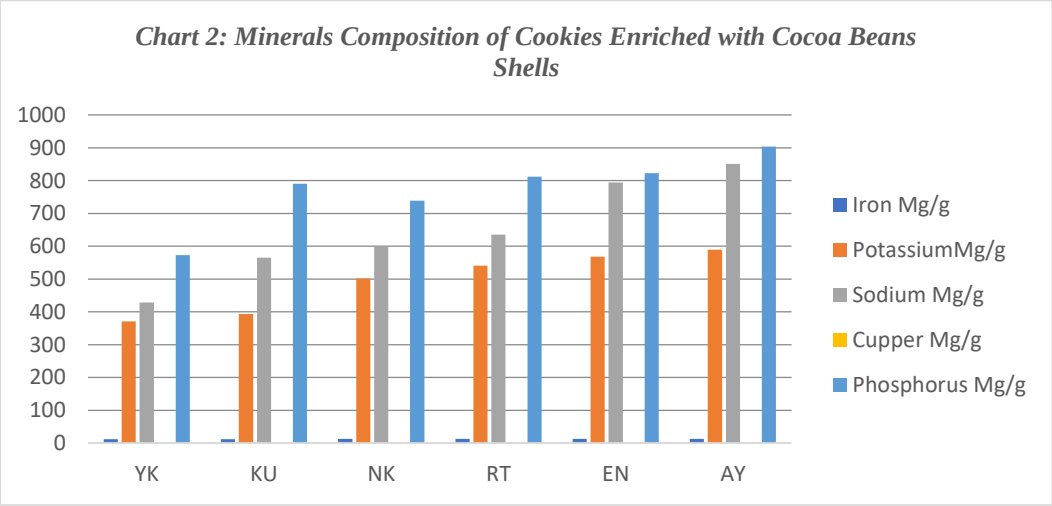

Phytochemicals are biologically active compounds, found in plants in small amounts, although they are not established nutrients but they contribute significantly to protection against degenerative disease (Dreosti, 2000). The phytochemical composition of the enriched cookies indicates that flavonoids which is known to have protective effects which includes anti-inflammatory, anti-oxidants, antiviral and anticarcinogenic properties (Rosin et al., 2019; Neshatdoust et al., 2016; Vauzour et. al., 2010; Middleton *et. al*., 2000) has the largest values while caffeine recorded the least figures. The phytochemical contents were found to increase as the percentage inclusion of CBS increases as shown in table 4. Similar increase in polyphenolic compounds was observed in biscuits supplemented with bambara groundnut and orange peel when compared to 100% whole wheat cookies (Adefegha and Oboh, 2013). Tannin content of the cookies range from 1.17 to 1.49mg/g which is a reasonable small quantity. Total phenolic content recorded is in the range of 0.32 to 0.39mg/g while caffeine is between 0.15 to 0.16mg/g. The contribution of cocoa beans shell to flavonoid composition of the cookies is higher than the other phytochemicals as it is shown in the range of 1.41 to 3.18mg/g. The total phenol content in the cocoa beans shells containing cookies is less than the total phenol value of 4.01 mg/g reported for chocolate cookies (Stahl et al., 2009). This is understandable since the shells of a cocoa constitutes approximately 12-15% of the total mass of the beans (Chronopoulos et al., 2011). The flavonoid content of the cookies ranged from 1.41 to 3.10 mg/g. The control sample (YK) also had the least flavonoid content when compared to the enriched cookies

Table 4. Phytochemical Composition of Cookies Enriched with Cocoa Bean Shell

Sample	Tannin (mg/g)	Total Phenolics (mg/g)	Flavonoids (mg/g)	Caffeine (mg/g)
YK	1.17±0.01^{a}	0.32±0.01^{c}	1.41±0.04^{f}	0.15±0.00^{c}
KU	1.19±0.01^{a}	0.34±0.00^{b}	1.64±0.05^{e}	0.15±0.00^{c}
NK	1.28±0.03^{a}	0.35±0.00^{b}	1.93±0.04^{d}	0.15±0.00^{c}
RT	1.29±0.04^{a}	0.37±0.00^{a}	2.31±0.04^{c}	0.16±0.00^{c}
EN	1.3±0.41^{a}	0.38±0.00^{a}	2.42±0.06^{b}	0.16±0.00^{ab}
AY	1.45±0.01^{a}	0.39±0.02^{a}	3.1±80.04^{a}	0.16±0.00^{a}

Means with different superscripts within the same column are significantly different ($p < 0.05$)
YK=0g CBS, KU= 2g CBS, NK=4g CBS, RT=6g CBS, EN=8g CBS and AY= 10g CBS.

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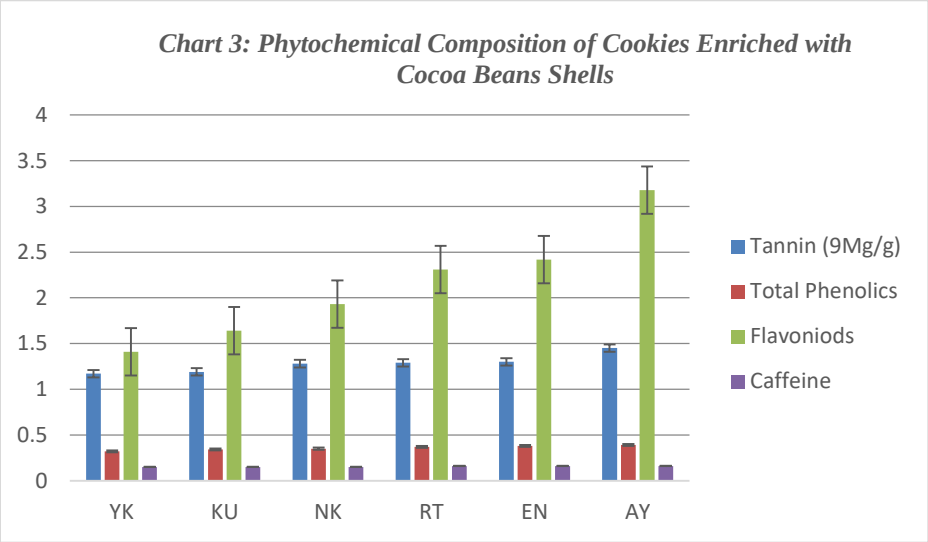

Another important quality factor is the evaluation of microbial load of the enriched cookies to ensure its safety. Total bacteria count (Table5) of $3.1x10^3$ cfu/g was detected for control as against $3.0x10^2$ and $1.7x10^2$ cfu/g for samples NK and EN while no bacteria were detected for samples KU, RT and AY after culturing in nutrient agar. There was no detection of any enteric bacilli generally known as feacal coliforms (*Escherichia coli* and *Salmonella*) after culturing in eosin methylene blue agar in any of the samples. Cocoa beans are fermented, dried, and stored most commonly in unhygienic conditions where the beans could absorb moisture and other intrinsic parameters that can encourage mold growth. Copeti et al., (2012) and Okiyama et al. (2017), reported that *Aspergillus* and *Penicillium* are fungi mostly found on cocoa beans and are most concentrated in the cocoa shells. For molds, *Aspergillus flavus* was the only mold detected in the control sample (YK), it was nor found in any of the enriched samples. The percentage of *A. ochraceus* increased with increase in the quantity of CBS in the samples. A. carbonarius was also detected in samples KU and NK, although, if phased-out as CBS concentration increases. Both Fusarium and Rhizopus were detected at lower percentages after culturing on Potato dextrose agar for 72 hours at room temperature. This contamination may have been brought about through handling by the processor, however, the highest total plate count recorded is still within the permissible limits of 2.0 $x10^5$cfu/g standard set by the WHO (1994), also for yeast and mold is < 1.0 x104 cfu/g for baked products (cake, bread and biscuits). It can be concluded that the enriched cookies are safe for consumption since it had a lower microbial profile than the WHO Standard of 1994.

Table 5: Microbiological Evaluation of Cookies Enriched with CBS

TOTAL BACTERIA COUNTS						
	Samples (cfu/g)					
Culture Media	**YK**	**KU**	**NK**	**RT**	**EN**	**AY**
Nutrient Agar	$3.1x10^3$	ND	$3.0x10^2$	ND	$1.7x10^2$	ND
Eosin Methylene Blue Agar	ND	ND	ND	ND	ND	ND
MOLDS	**TOTAL MOLDS COUNT (%)**					
Aspergillus flavus	ND	ND	ND	ND	ND	ND
Aspergillus ochraceous	ND	41.67	46.15	66.67	83.33	ND
Aspergillus carbonarius	ND	58.33	38.46	ND	ND	ND
Fusarium spp.	ND	ND	ND	33.33	16.67	ND
Rhizopus stolonifer	ND	ND	15.38	ND	ND	ND

NOTE: ND (Not Detected), YK=0g CBS, KU= 2g CBS, NK=4g CBS, RT=6g CBS, EN=8g CBS and AY= 10g CBS.

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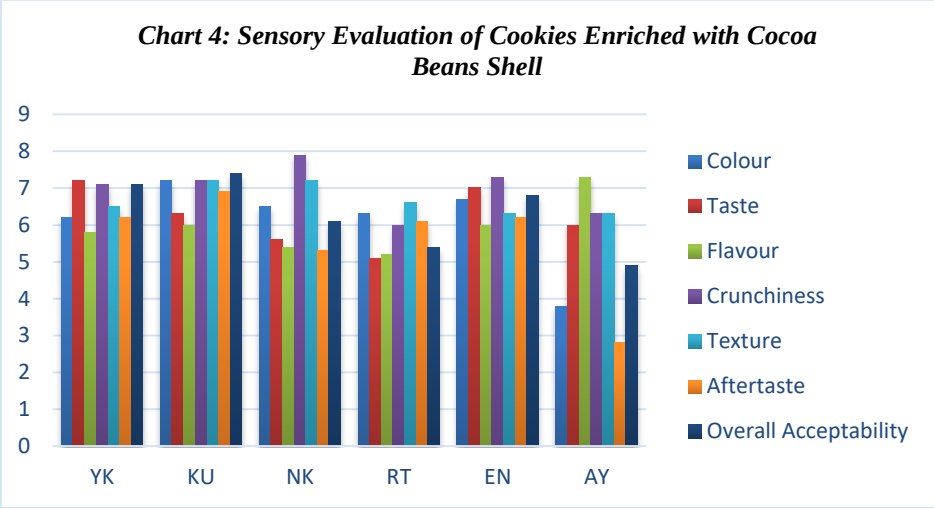
Table 6: Sensory Evaluation of CBS Enriched Cookies

	Attributes						
Samples	**Colour**	**Taste**	**Flavour**	**Crunchiness**	**Texture**	**Aftertaste**	**Overall Acceptability**
YK	6.2000^{a}	7.2000^{a}	5.8000^{ab}	7.1000^{ab}	6.5000^{a}	6.2000^{a}	7.1000^{ab}
KU	7.2000 ^{a}	6.3000^{ab}	6.0000^{ab}	7.2000^{ab}	7.2000^{a}	6.9000^{a}	7.4000^{a}
NK	6.5000^{a}	5.6000^{ab}	5.4000^{b}	7.9000^{a}	7.2000^{a}	5.3000^{a}	6.1000^{abc}
RT	6.3000^{a}	5.1000^{b}	5.2000^{b}	6.0000^{b}	6.6000^{a}	6.1000^{a}	5.4000^{bc}
EN	6.7000^{a}	7.0000^{a}	6.0000^{ab}	7.3000^{ab}	6.3000^{a}	6.2000^{a}	6.8000^{ab}
AY	3.8000^{b}	6.0000^{ab}	7.3000^{a}	6.3000^{b}	6.3000^{a}	2.8000^{b}	4.9000^{c}

Samples with different superscripts within the same column were significantly different ($p<0.05$).
YK=0g CBS, KU= 2g CBS, NK=4g CBS, RT=6g CBS, EN=8g CBS and AY= 10g CBS.



CONCLUSSION

The inclusion of cocoa beans shell in the production of cookies has shown improvement in the nutritional compositions, minerals values, and the phytochemical constituents of the enriched cookies. This addition has greatly impacted on the sensorial attributes like colour, taste. The flavour, crunchiness, texture and overall acceptability of the produced cookies. Although, sample AY with the highest addition of CBS (10g) is poorly rated in terms of colour and aftertaste but it is highly acceptable for all other attributes. Cocoa beans shells has interesting characteristics of strong brown colour and chocolate flavour which enables its application as a natural colourant and as flavouring agent. Its contribution to the phytochemical consitituents of the cookies has made it a functional ingredient of great value. The use of cocoa beans shells has proved to be a promising ingredient as a food enricher and for other nobler purposes to reduce waste generation, thus, contributing to reduction in waste management costs and create a clean environment for chocolate and other cocoa derivatives in the food industries.

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