

Assessment of Nutritional Potential and Phytochemical Constituents of Cocoyam (*Xanthosoma sagittifolium* spp.) /Wheat Flour Composite BreadRichard A. Ajani¹, Sefiat I. Hamzat², Racheal O. Adeyi³, Micheal A. Ajamu⁴, Wasiu O. Alabi⁵ and Adekunle O. Adeoye^{6*}^{1,23}Department of Biochemistry, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.⁴Department of Anatomy, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.⁵Department of Animal Production and Health, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.⁶Department of Food Science, Ladoké Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.**ABSTRACT**

Cocoyam (*Colocasia esculenta*), a lesser-known root crop, is rich in dietary fibers, essential vitamins, and has been identified as a potential candidate for enhancing the nutritional profile of conventional wheat-based bread. This study sought to investigate the nutritional compositions and phytochemical properties of the composite bread produced from cocoyam (CYF) and wheat flour (WF) *in vitro*. The CYF and WF composite bread was prepared in various proportions of 100:0 (100% CYF), 50:50 (50% CYF:50% WF), 20:80 (20% CYF:80% WF), 10:90 (10% CYF:90% WF) and 0:100 (100% WF), and flavored with 2g of cocoa powder for the production of composite bread. The bread samples were investigated for calorific value, sensory evaluation, proximate analysis, mineral composition and phytochemicals constituents. The results revealed significant ($p < 0.05$) decrease in the calorific value as compared to the control. However, sensory evaluation revealed a significant ($p < 0.05$) increase in the aroma but no significant ($P > 0.05$) difference in its taste, texture, colour and its general acceptability as compared to the control (100% WF bread). The proximate composition result revealed a significant increase in the moisture content of 100% CYF bread (22.39 ± 0.07) as compared to the control, 100% WF bread (18.15 ± 0.13). More so, the results of the K, Mg, Fe and Ca revealed significant ($p < 0.05$) increase as compared to the control bread. Hence, it could be deduced that the bread produced from cocoyam / wheat flour blend could be a good source of antioxidants and also would be a recipe for patients with nutritional challenges.

Keywords: Cocoyam flour, Proximate analysis, Calorific value, Phytate and cocoa powder.

Received 01 May 2025

Revised 29 May 2025

Accepted 30 May 2025

Published online 01 July 2025

Copyright: © 2025 Ajani *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.**Introduction**

Cocoyam is predominantly cultivated in tropical regions, where it is valued mainly for its edible corms or roots, as well as its leaves. It holds significant importance as a staple food crop across various regions, particularly in the Pacific Islands, Asia, and Africa.^{1,2} This widespread reliance on cocoyam can be attributed to several advantages it offers over other root or tuber crops. Notably, cocoyam possesses a longer shelf life, has a remarkable ability to thrive under adverse environmental conditions, and serves as an affordable and accessible source of energy in the diets of economically disadvantaged rural populations.³ There are two principal species of cocoyam that are widely cultivated: taro (*Colocasia esculenta*) and tannia (*Xanthosoma* species). Interestingly, the Roots, Tubers and Bananas Newsletter⁴ opined that Africa stands out as the leading global producer of cocoyam. Explicitly, within Africa, Nigeria leads production with an annual output of approximately 5.49 million metric tons, accounting for about 45.90% of the total global production.

However, the Food and Agriculture Organization (FAO)⁵ presents a slightly different figure, estimating Nigeria's output at 3.46 million metric tons, representing 37.00% of the global total. China and Ghana follow Nigeria in production levels, contributing 1.64 million metric tons and 1.51 million metric tons, respectively.⁶ Cocoyam offers considerable nutritional benefits when compared to another root and tuber crops.⁷ It is notably richer in crude protein and contains starch that is highly digestible due to the fine structure of its starch granules. Additionally, it provides significant amounts of calcium, phosphorus, vitamin A, and several B-vitamins.^{8, 7, 9} The crop also boasts a superior phytonutrient profile, comprising dietary fibre and antioxidants, while also being naturally gluten-free.¹⁰ Among its nutritional highlights is the presence of essential B-complex vitamins such as pyridoxine (vitamin B6), folate, riboflavin, pantothenic acid, and thiamine. Its corms are particularly rich in carbohydrates, containing between 70% and 80% on a dry weight basis, primarily in the form of starch.^{11, 12, 13} This composition not only lends desirable functional properties to food products but also contributes to energy supply and promotes a feeling of fullness or satiety.¹⁴ When compared nutritionally to other staple root crops like cassava and yam, cocoyam is superior in terms of protein, mineral, and vitamin contents. Moreover, it has a higher proportion of digestible starch.^{7, 15, 16} The leaves of cocoyam also provide notable nutritional benefits. They contain significantly higher levels of protein than those of other root and tuber crops and are enriched with essential minerals such as calcium, phosphorus, and iron. Furthermore, they supply vital vitamins including vitamin C, thiamin, riboflavin, and niacin.^{15, 2} Due to these attributes, cocoyam has been recommended for a variety of dietary needs, including for diabetic patients, elderly individuals, children with food allergies, and people suffering from intestinal issues.^{17, 6, 2} The high concentrations of beta-carotene, iron, and folic

*Corresponding author. Email: aoadeoye@lautech.edu.ng

Tel: +08036976365

Citation: Ajani RA, Hamzat SI, Adeyi RO, Ajamu MA, Alabi WO, Adeoye AO. Assessment of Nutritional Potential and Phytochemical Constituents of Cocoyam (*Xanthosoma sagittifolium* spp.) /Wheat Flour Composite Bread. Trop J Phytochem Pharm Sci. 2025; 4 (6): 243 – 254 <http://www.doi.org/10.26538/tjpps/v4i6.1>

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

acid in cocoyam leaves are particularly beneficial, offering protection against conditions such as acidosis and kidney stone formation.¹⁸ Despite its many benefits, cocoyam remains underutilized in Nigeria. Its use is largely restricted to subsistence farming, and it is considered a neglected crop despite its potential to improve nutrition and food security.² All plants, including fruits, vegetables, legumes, and grains, naturally produce phytochemicals. These compounds are integral to a plant's immune defense system, providing protection against threats such as viruses, bacteria, fungi, and parasites. Remarkably, these phytochemicals can also offer similar protective benefits to humans. While some phytochemicals qualify as vitamins, their primary role is to safeguard the body's cells from damage caused by environmental toxins and the body's own metabolic processes.¹⁹ Phytochemical analyses of cocoyam roots indicate strong antioxidant properties, which help reduce the risk of various diseases by neutralizing free radicals that could otherwise cause DNA damage.¹⁹ In an effort to reduce Nigeria's dependency on large-scale wheat imports, researchers have advocated for the adoption of composite flours in bread production.²⁰ This approach involves partially replacing wheat flour with locally sourced alternatives, such as cocoyam flour, to promote the use of indigenous crops. The aim is not only to reduce the national demand for imported wheat but also to stimulate local agriculture and the utilization of underexploited domestic resources.²¹ Bread, one of humanity's oldest and most widely consumed foods, has seen rising consumption patterns globally and in Nigeria. While it was not traditionally a staple in daily Nigerian diets, shifting population demographics and evolving dietary preferences have led to its increased popularity over recent decades.²² Bread is now universally recognized for its convenience and nutritional value, offering an array of essential macronutrients which include carbohydrates, proteins, and fats along with critical vitamins and minerals that enrich the sustenance of human health.²³ To further decrease reliance on wheat imports and enhance the nutritional profile of bread, efforts have been made to incorporate flours derived from high-protein seeds and locally cultivated crops into bread-making. This not only curtails the demand for imported wheat but also facilitates the production of protein-enriched bread varieties.²⁴ Therefore, this research was designed to evaluate the energy values, nutritional potential and phytochemical constituents of composite bread produced from cocoyam (CYF) / wheat flour (WF) blends flavoured with cocoa powder.

Materials and Methods

Materials

Cocoyam used for this study was sourced from a local market located in Elekokan Market, situated within the Igangan area of Ibarapa Local Government Area in Oyo State, Nigeria. This location lies at a geographical coordinate of approximately Latitude 7.68° North and Longitude 3.18° East. Upon procurement, the cocoyam corms were subjected to preliminary processing which involved peeling, thoroughly washing them to remove dirt and impurities, and subsequently cutting them into smaller, manageable pieces. These pieces were then oven-dried using a cabinet dryer maintained at a constant temperature of 45°C over a period of 72 hours to ensure complete moisture removal. Once adequately dried, the samples were milled into fine flour suitable for experimental use. In addition to cocoyam, other ingredients required for the study were sourced from Waso Market, a prominent commercial hub located in Ogbomoso city, also in Oyo State. These included wheat flour (specifically Golden Penny brand), cocoa powder, yeast, and eggs, all of which were purchased to maintain consistency and standard in formulation. Dates (*Phoenix dactylifera*), employed in this research as a natural sweetening agent in place of artificial sweeteners, were also obtained from the same market. All chemical reagents utilized in the various stages of analytical procedures were of analytical grade to ensure precision and reliability of results. Furthermore, all water used throughout the experimentation and preparation processes was glass-distilled to eliminate any possible contaminants that could interfere with the accuracy of the analysis.

Production of cocoyam-wheat composite flour bread

The cocoyam tubers underwent a sequence of preparatory steps prior to utilization in flour production. Initially, they were peeled to remove the outer skin, after which they were thoroughly washed with clean water to eliminate all traces of dirt and impurities. The cleaned cocoyam was then cut into smaller, uniform-sized pieces to facilitate even drying. These pieces were carefully arranged and dried in a cabinet dryer, maintained at a constant temperature of 45°C, for a duration of 72 hours to ensure complete dehydration. Once fully dried, the cocoyam pieces were milled using a mechanical grinder to obtain a fine powder referred to as cocoyam flour (CYF). Following the preparation of the cocoyam flour, a series of composite flour mixtures were formulated by blending the cocoyam flour (CYF) with wheat flour (WF) in varying proportions. The mixing ratios included 100:0 (representing 100% cocoyam flour and 0% wheat flour), 50:50 (50% CYF and 50% WF), 20:80 (20% CYF and 80% WF), 10:90 (10% CYF and 90% WF), and 0:100 (0% CYF and 100% WF), respectively. These different combinations were prepared to evaluate the performance of cocoyam flour in partial and complete substitution of wheat flour in bread production. To sweeten the dough mixtures naturally, dates (*Phoenix dactylifera*) obtained from Waso Market in Ogbomoso were incorporated. Egg albumin was employed as an emulsifying agent to improve texture and structural stability of the bread. Cocoa powder was added not only to impart a pleasant flavour to the final product but also to serve as an additional source of phenolic compounds, enhancing the antioxidant profile of the bread. Yeast was included as the leavening agent to promote dough rise, while water was added in appropriate quantities to achieve the desired dough consistency and palatability. The prepared dough samples were then baked using a gas oven (Nexus model IX 9000, manufactured in Europe). Baking was conducted at a temperature of 250°C for a period of 30 minutes or until the surface of the bread developed a golden-brown colour, indicating that it was fully baked and ready for evaluation.

Methods

Determination of Calorific Value of Composite Bread

The calorific value of the composite bread was determined using the method of Bomb Calorimetry (ASTM D240-17) by the method of AOAC.²⁵ A small sample (1-2g) of the composite bread was placed in a sealed container or bomb calorimeter filled with oxygen, and the sample was ignited. The heat released during combustion was measured, allowing for the calculation of the calorific value expressed in kilojoules per gram (KJ/g). Multiple samples were tested to ensure accuracy and reproducibility, and the average calorific values were reported alongside standard deviations (equation 1).

Gross Calorific value (KJ/kg) =

Mass of water × specific heat of water × temperature rise / mass of fuel burned equation 1

Sensory evaluation of the composite bread

The sensory evaluation of the composite bread samples was conducted following the procedure outlined by Potter.²⁶ This evaluation aimed to determine consumer acceptability of the bread variants through organoleptic assessment. A panel consisting of 25 final-year students from the Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria, was selected to serve as evaluators. These students were briefed on the evaluation criteria and instructed to independently assess all the different composite bread samples prepared for the study.

Each of the 25 student panellists was asked to assess and record their level of preference for five key sensory attributes: aroma, taste, texture, colour, and overall acceptability. To ensure uniformity and standardization in data collection, a 7-point Hedonic Scale was used. The scale ranged from 7 to 1, where a score of 7 represented "excellent," 6 indicated "very good," 5 denoted "good," 4 stood for "average," 3 for "fair," 2 for "poor," and 1 signified "very poor."

Panellists were required to evaluate each sample independently and provide a rating for each sensory attribute based on their personal perception and preference. The data obtained from all panellists were then compiled and subjected to statistical analysis. The mean scores for each sensory attribute were calculated using the method described by Deliza and Macfie²⁷ to determine the overall acceptability and quality ranking of each composite bread formulation.

Proximate compositions of the composite bread

The proximate compositions of the composite bread samples formulated from cocoyam-wheat flour and enriched with cocoa powder were analyzed to determine their nutritional content. The analyses were carried out in accordance with the standardized procedures outlined by the Association of Official Analytical Chemists (AOAC).²⁵ These methods were employed to ensure accuracy, reliability, and consistency of the analytical results.

Prior to the commencement of the analysis, all composite bread samples were subjected to a preparatory step in which they were ground into fine powder using an electric blender. This homogenization process was essential to ensure that the samples were uniform in texture and composition, thereby enabling precise measurement of their nutritional parameters. The proximate analysis included the determination of moisture content, crude protein, crude fat, ash content, crude fibre, and carbohydrate content by difference. Each of these components was measured according to the guidelines stipulated in the AOAC methods,²⁵ which are widely recognized and adopted for food composition analysis in research and industry settings. This analytical procedure provided a comprehensive nutritional profile of the cocoyam-wheat composite breads, enabling the assessment of how the inclusion of cocoyam flour and cocoa powder influenced the macronutrient composition of the final baked products.

Determination of moisture content

The moisture content of the composite bread samples was determined using the standard air-oven dry method, a widely accepted technique for assessing the water content in food products. This procedure involved accurately weighing two grams (2 g) of each composite bread sample using a high-precision analytical balance to ensure measurement accuracy. The weighed samples were then placed in clean, dry crucibles and transferred into a hot air oven pre-set to a temperature of 105°C.

The bread samples were subjected to continuous drying in the oven for a period of four (4) hours to facilitate the complete removal of moisture content. Upon completion of the initial drying period, the samples were carefully removed from the oven and allowed to cool in a desiccator. The use of a desiccator was essential to prevent moisture reabsorption from the surrounding environment, thereby preserving the integrity of the drying process.

After cooling, the samples were reweighed, and the drying process was repeated several times at 105°C until a constant weight was achieved. This ensured that all residual moisture had been completely eliminated, confirming the final dry mass of the sample. The moisture content was subsequently calculated and expressed as a percentage of the initial sample weight. The formula used for determining the moisture content is presented (equation 2):

$$\text{Moisture content (\%)} = \frac{\text{Loss in Weight during drying}}{\text{Weight of the sample}} \times 100 \dots\dots\dots \text{equation 2}$$

Determination of Ash content

The ash content of the composite bread samples was determined using the dry ashing method with the aid of a hot muffle furnace. This method is commonly used to estimate the total mineral content in food samples by incinerating organic matter. Precisely two grams (2 g) of the finely ground composite bread sample were weighed using a calibrated analytical balance and transferred into a clean, pre-weighed porcelain crucible. The crucible containing the sample was then placed in a hot muffle furnace and subjected to ignition at a high temperature

of 550°C. The incineration process was carried out for a continuous period of five (5) hours to ensure complete combustion of all organic materials present in the sample. During this process, the organic components were vaporized, leaving behind a white or grey ash residue which represents the inorganic mineral content of the bread.

After the heating period, the crucible was carefully removed from the furnace using heat-resistant tongs and allowed to cool in a desiccator. Cooling in a desiccator was necessary to prevent moisture from being reabsorbed by the ash, which could compromise the accuracy of the measurement. The weight of the crucible and the residual ash was then recorded. Ashing was considered complete once a constant weight was achieved, confirming that all combustible material had been incinerated. The percentage ash content of the sample was then calculated based on the initial sample weight and the final weight of the ash using the formula provided below (equation 3):

$$\text{Ash content (\%)} = \frac{\text{Weight of the Ash}}{\text{Weight of the sample}} \times 100 \dots\dots\dots \text{equation 3}$$

Determination of crude protein content

The crude protein content of the composite bread samples was determined using the standard micro-Kjeldahl method, a reliable and widely employed technique for the quantitative determination of nitrogen, which is then converted to protein content. This method is based on the principle that the nitrogen present in the organic compounds of the sample is converted to ammonium sulfate under acidic conditions, followed by distillation and titration. To begin the procedure, precisely two hundred milligrams (200 mg) of the finely ground bread sample were accurately weighed and transferred into a clean micro-Kjeldahl digestion flask. To facilitate the digestion process, four hundred milligrams (400 mg) of a Kjeldahl catalyst mixture; consisting of sodium tetraoxosulfate (VI) and copper (II) tetraoxosulfate (VI) were added to the flask along with 3.5 milliliters (mL) of concentrated sulfuric acid (H₂SO₄). This mixture promotes the breakdown of organic matter and the release of nitrogen in the form of ammonium ions.

The flask was then placed on an electric heating unit and the mixture was digested for approximately two (2) hours, or until a clear, colourless solution was obtained. This indicated the complete digestion of organic matter. After cooling, the digest was carefully transferred into a distillation apparatus. In the distillation step, 20 mL of 40% sodium hydroxide (NaOH) solution was added to the digest in order to neutralize the acid and liberate ammonia gas from the ammonium sulfate. The liberated ammonia gas was then distilled and captured in a receiving conical flask containing 10 mL of 2% boric acid solution. This setup allowed the ammonia to react with the boric acid, forming a weakly basic solution.

The amount of nitrogen present was finally determined by titrating the borate-ammonia complex with a standard 0.02 M hydrochloric acid (HCl) solution. A mixed indicator solution of bromocresol green and methyl red, dissolved in alcohol, was used to detect the endpoint of the titration. The volume of acid consumed in the titration was used to calculate the nitrogen content of the sample, which was subsequently converted to crude protein by multiplying by the appropriate nitrogen-to-protein conversion factor. The crude protein content was expressed using the formula (equation 4):

$$\text{Crude Protein (\%)} = \frac{\text{Molarity of HCl} \times 0.014 \times \text{Titre value} \times \text{dilution factor}}{\text{Weight of the sample}} \times 10 \dots\dots\dots \text{Equation 4}$$

Determination of crude fat content

The crude fat content was determined according to the method described by AOAC,²⁵ using a Soxhlet extractor apparatus. A quantity of 2 grams of the bread sample was accurately weighed and carefully placed in filter paper, which was then inserted into an extraction thimble. Petroleum ether, with a boiling point range of 40–60°C, served as the solvent for the fat extraction process, which was carried out over a period of 5 hours to ensure thorough extraction of the fat

content. After the extraction process was completed, the filter paper containing the sample was removed from the extraction thimble and transferred to a drying oven set at 106°C. It was left in the oven for 30 minutes to eliminate any residual solvent. Following the drying stage, the sample was allowed to cool in a desiccator to prevent moisture absorption. Once cooled, the sample was weighed again to determine the amount of crude fat extracted by calculating the difference in weight. The crude fat was calculated thus (equation 5):

$$\text{Crude fat (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of the sample (g)}} \times 100 \text{ ...equation 5}$$

Determination of crude fibre content

The crude fibre content of the composite bread and control bread samples was analyzed following the standard procedure described by the Association of Official Analytical Chemists (AOAC).²⁵ To begin the analysis, precisely, 2 grams of the composite bread sample (initial weight denoted as W_0) was accurately weighed using an analytical balance. This sample was placed into a 500 mL conical flask, and 200 mL of freshly prepared diluted sulfuric acid (H_2SO_4) at a concentration of 1.25% was added. The mixture was then subjected to gentle boiling for a duration of 30 minutes until a constant volume was achieved, ensuring adequate breakdown of soluble components. After boiling, the hot mixture was filtered using a muslin cloth, which effectively separated the insoluble residue from the liquid. The residue was thoroughly washed with distilled water to remove all traces of acid. Subsequently, 200 mL of 1.25% sodium hydroxide (NaOH) solution was added to the residue. This mixture was also boiled for 30 minutes, promoting further breakdown of digestible materials. Following the second boiling step, the mixture was again filtered through a muslin cloth and washed with hot distilled water four times to eliminate any residual sodium hydroxide. The washed residue was then subjected to additional rinsing with 10% hydrochloric acid (HCl), followed by another four washes with hot distilled water to neutralize the pH and remove any remaining chemical traces. The neutralized residue was further rinsed three times with ethanol and three times with petroleum ether (boiling point range of 40–60°C) to remove fats and other interfering substances. After this extensive washing, the treated residue was carefully transferred into a clean, dry silica crucible. The crucible was placed in an electric oven and dried at a constant temperature of 105°C until a stable weight (W_1) was recorded. It was then allowed to cool in a desiccator to prevent moisture absorption from the atmosphere.

The next step involved ignition of the dried residue in a muffle furnace set at 600°C for a duration of 30 minutes to burn off all organic matter, leaving only ash. The crucible was removed from the furnace, cooled in a desiccator, and weighed again to obtain the final weight (W_2). The crude fibre content was calculated by subtracting ash weight from weight of the dried residue, and the value was expressed as a percentage of the original sample weight. The crude fibre was calculated as follows (equation 6):

$$\text{Crude fibre (\%)} = \frac{W_1 - W_2}{W_0} \times 100 \text{equation 6}$$

Evaluation of carbohydrate content of the composite bread

The carbohydrate contents of the composite bread samples were determined using the difference method based on their dry weight composition. In this method, the individual proximate components namely, moisture content, crude fat, crude fibre, ash content, and crude protein were first quantified and their respective percentage values obtained from the proximate analysis. These components collectively account for the major constituents of the bread samples, excluding carbohydrates. To calculate the carbohydrate content, the sum of the percentages of moisture, crude fat, crude fibre, ash, and crude protein was subtracted from 100%. The remaining portion was attributed to carbohydrate content. This approach assumes that the other components make up the total dry matter, and the difference provides an accurate estimation of the carbohydrate concentration in the bread. The final carbohydrate content was expressed as a percentage on the basis of the sample's dry weight.

Determination of mineral Contents

An appropriate hollow cathode lamp was selected and installed in the Atomic Absorption Spectrophotometer (AAS) based on the specific element or mineral to be analyzed. The AAS instrument was properly configured and allowed to stabilize using a standard solution to ensure accurate calibration and reliable readings. The stock standard solution prepared for each mineral had a concentration of 10 ppm (parts per million), which served as the reference point for subsequent measurements.

Once the instrument was stabilized, the standard solution for each metal was aspirated into the flame of the spectrophotometer to establish a baseline absorbance value. Following this, the prepared sample solutions were also aspirated into the flame, and the corresponding concentrations of each mineral were determined by comparing the sample absorbance readings with those of the standards. The absorbance values obtained from the standards were used to calculate the concentrations of the minerals in the samples. These concentrations were expressed in milligrams per 100 grams (mg/100g) of the sample, and all values were derived in accordance with the established procedures.²⁵

Qualitative Analysis of Phytochemical compounds

The preliminary phytochemical screening of the extracts of each composite bread was carried out using the method as recorded by Altamimi et al.²⁸

Determination of total Alkaloids

This procedure was carried out following the method described by Shamsa, et al.²⁹ To prepare the bromocresol green solution, exactly 69.8 mg of bromocresol green powder was measured and heated gently with 3 mL of a 2N sodium hydroxide (NaOH) solution. After partial dissolution, 5 mL of distilled water was added to facilitate complete dissolution of the dye. Once fully dissolved, the solution was transferred into a volumetric flask and diluted to a final volume of 1000 mL using distilled water.

A phosphate buffer solution with a pH of 4.7 was also prepared. This was done by first dissolving 71.6 g of sodium phosphate (Na_2HPO_4) in 1000 mL of distilled water to form a 2M solution. The pH of this solution was then carefully adjusted to 4.7 using a citric acid solution, which was prepared by dissolving 42.02 g of citric acid in 1000 mL of distilled water.

To prepare the atropine standard solution, 1 mg of pure atropine (sourced from Sigma Chemical, USA) was accurately weighed and dissolved in 10 mL of distilled water. For the sample analysis, the extracts were collected in a 10 mL volumetric flask, and then diluted as necessary to reach the 10 mL mark using chloroform. The absorbance of the resulting atropine-bromocresol green complex in chloroform was then measured at a wavelength of 470 nm using a spectrophotometer (BIOBASE BK-V/1000), manufactured in China.

Determination of tannin content

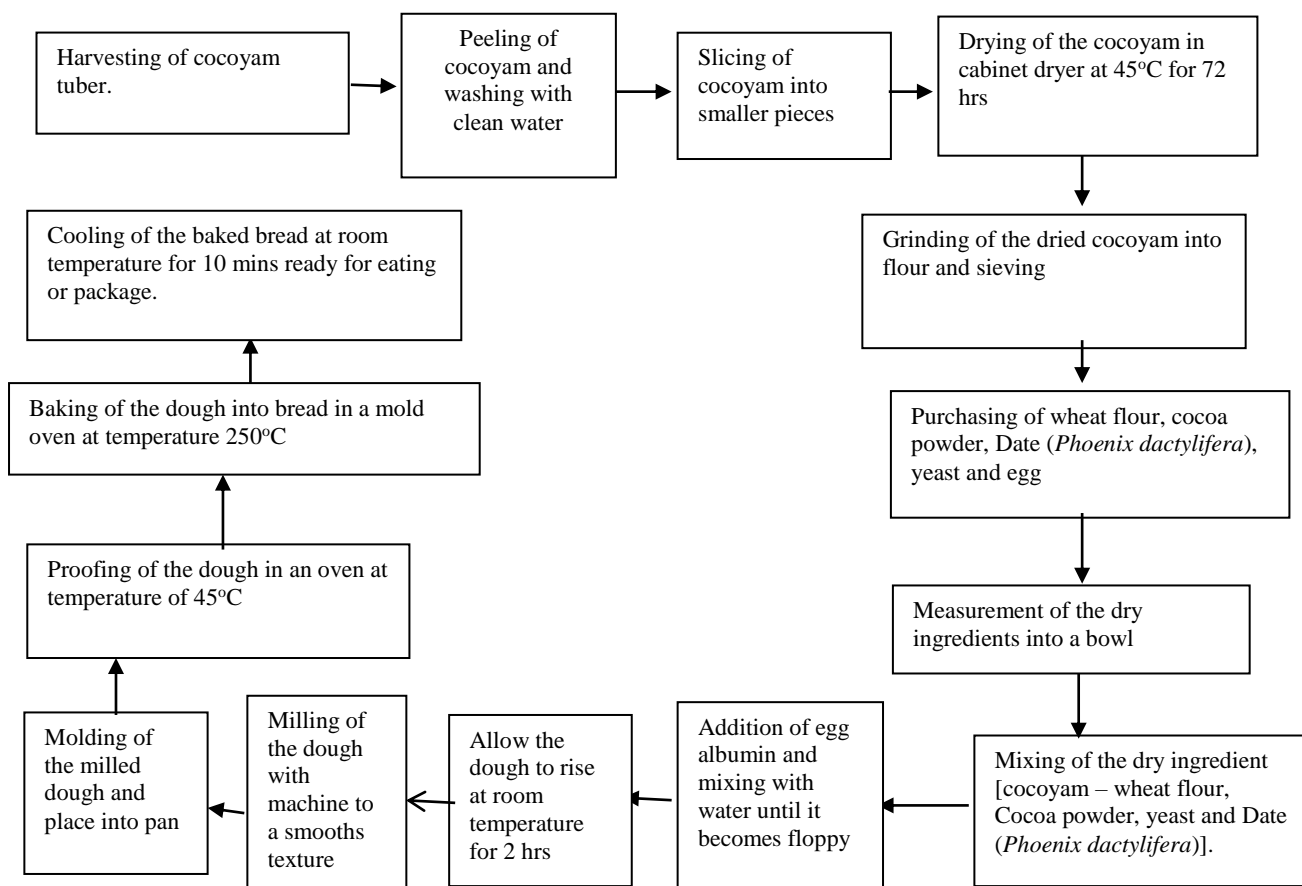
This analysis was carried out using the method described by Medoua et al.³⁰ Precisely two grams (2 g) of each sample were accurately weighed and transferred into a 250 mL conical flask. To the sample, 200 mL of 0.004 M potassium ferricyanide [$K_3Fe(CN)_6$] solution was added, followed by the addition of 10 mL of 0.008 M ferric chloride ($FeCl_3$) prepared in 0.008 M hydrochloric acid (HCl). The reaction mixture in the flask was then allowed to stand undisturbed for 20 minutes to ensure proper interaction of the reagents with the sample. During this time, the contents were gently stirred at 10-minute intervals to facilitate thorough mixing and reaction. After 20 minutes, a 1 mL aliquot of the reaction mixture was withdrawn using a pipette. This aliquot was then treated with 2 mL of 0.008 M ferric chloride ($FeCl_3$) in 0.008 M hydrochloric acid (HCl), followed by the addition of 10 mL of 0.0015 M potassium ferricyanide [$K_3Fe(CN)_6$]. After the final reagent was added, the mixture was allowed to stand for 30 seconds to complete the colour development. The absorbance of the resulting solution was then measured at a wavelength of 720 nm using a spectrophotometer (BIOBASE BK-V/1000), manufactured in China, with a reagent blank serving as the reference.

Table 1: Composition of the composite bread

Composite Bread ingredients	Percentage (g/100g)
Composite flour	75.00
Dates (<i>Phoenix dactylifera</i>)	4.00
Egg Albumin	3.00
Cocoa Powder	2.00
Yeast	2.00
Water	14.00

KEY:

A = 100% CYF bread + FL (100% cocoyam flour bread + cocoa powder).
 B = 50% CYF + 50% WF bread + FL (50% cocoyam flour + 50% wheat flour bread + cocoa powder).
 C = 20% CYF + 80% WF bread + FL (20% cocoyam flour + 80% wheat flour bread + cocoa powder).
 D = 10% CYF + 90% WF bread + FL (20% cocoyam flour + 80% wheat flour bread + cocoa powder).
 E = 100% WF bread + FL (100% WF bread + cocoa powder).
 X = 100% WF bread (100% wheat flour bread without cocoa powder).
 Y = 100% CYF bread (100% cocoa powder bread without cocoa powder).

**Figure 2a:** Flow chart for the production of composite bread from cocoyam/wheat flour**Determination of phytate content**

The determination of phytate content in the composite bread samples was carried out following the method established by Wheeler and Ferrel.³¹ To begin the analysis, exactly 4 grams of each composite bread sample were accurately weighed and transferred into a beaker containing 100 milliliters of 2% hydrochloric acid (HCl). The mixture was thoroughly stirred to ensure proper dispersion and then allowed to soak for a duration of 3 hours at room temperature to facilitate the extraction of phytate compounds. After the soaking period, the solution was subjected to a two-step filtration process using double-layered filter paper in order to obtain a clear filtrate. From the resulting filtrate, exactly 25 milliliters were measured and poured into a clean conical flask. To this, 5 milliliters of 0.3% ammonium thiocyanate solution were added as an indicator reagent. Subsequently, 53.5 milliliters of distilled water were introduced into the flask to dilute the mixture appropriately. The prepared solution was then titrated against a standard iron (III) chloride solution. The iron (III)

chloride used contained a known concentration of 0.00195 grams of iron per milliliter. The titration process was carefully observed until a distinct brownish-yellow coloration appeared in the solution. This coloration signaled the endpoint of the titration and was noted to persist for a minimum duration of 5 minutes, confirming the presence and estimation of phytate content in the sample. The phytate content was expressed as mg/g phytate in the sample.

Statistical analysis

The results were reported as mean of replicate determination and standard deviation. Analysis of variance (ANOVA) and the least significant difference (LSD) were used to determine the confidence level at 95% using the Statistical package for Social Science (SPSS) statistical software, version 17.³²

Results and Discussion

Determination of Calorific value of the composite bread.

Table 2 expressed the result of calorific value using bomb calorimeter in Kilojoules/gram (KJ/g). The results of the composite bread revealed a significant ($p < 0.05$) decrease as compared to the control (100% WF bread). The trend of the values reveals that Commercial bread (13.56 ± 0.13^g KJ/g) > 100% WF bread (12.88 ± 0.17^f KJ/g) > 10% CYF + 90% WF bread + FL (11.29 ± 0.00^e KJ/g) > 20% CYF + 80% WF bread + FL (10.31 ± 0.15^d KJ/g) > 100% WF bread + FL (9.27 ± 0.01^c KJ/g) > 100% CYF bread + FL (8.96 ± 0.21^b KJ/g) > 100% CYF bread (8.56 ± 0.02^a KJ/g) > 50% CYF + 50% WF bread + FL (8.54 ± 0.04^a KJ/g). Calorific value is the amount of heat energy present in food or fuel and which is determined by the complete combustion of specified quantity at constant pressure and in a normal condition. It is also called calorific power or gross energy and it is expressed in kilojoule per kilogram (KJ/kg or KJ/g).³³ From the calorific value results, commercial bread had the highest calorific value (13.5 ± 0.13^g KJ/g) while 50% CYF + 50% WF bread + FL (8.54 ± 0.04^a KJ/g) had the least value. The calorific values of the bread samples indicate that the inclusion of cocoyam flour significantly reduces the energy content as compared to the commercial bread. This reduction in calorific value is beneficial for consumers seeking lower-calorie bread options. The addition of fortifying ingredients could slightly increase the calorific value, but the overall trend shows that higher proportions of cocoyam flour result in lower energy content.³⁴ This study highlights the potential of cocoyam flour as a valuable ingredient in producing nutritionally balanced, lower-calorie bread.



Figure 2b: Cocoyam- wheat composite bread

KEY:

A = 100% Cocoyam flour bread + cocoa powder, B = 50% Cocoyam flour + 50% wheat flour bread + cocoa powder, C = 20% Cocoyam flour + 80% wheat flour bread + cocoa powder, D = 10% Cocoyam flour + 90% wheat flour bread + cocoa powder, E = 100% Wheat flour bread + cocoa powder, X = 100% Wheat flour bread without cocoa powder, Y = 100% Cocoyam flour bread without cocoa powder.

Sensory evaluation of the composite bread

The result for the sensory evaluation is shown in Table 3. The sensory evaluation results of the cocoyam-wheat composite bread highlight the importance of taste, aroma, texture, color, and overall acceptability in determining consumer preferences. It was revealed that there was a significant ($p < 0.05$) difference in the aroma of the composite bread as compared with the control ranging from (4.28 -6.76). Taste revealed no significant ($p > 0.05$) difference from the control ranging from (3.36-4.48) of the composite breads except in 50% CYF + 50% WF bread + FL (3.36 ± 0.20^a) when compared 100% WF flour bread. This indicates that the replacement of wheat flour by cocoyam flour in bread is well accepted. Commercial bread received the highest scores for aroma (6.76 ± 0.18^e) and general acceptability (5.56 ± 0.22^d), suggesting that consumers may favor traditional bread for its familiar sensory attributes.³⁵ In comparison, the 100% cocoyam flour bread had lower ratings across all sensory categories, particularly in taste and texture, indicating that the absence of wheat may contribute to a less favorable sensory experience.³⁶ The 50% CYF and 50% WF

bread + FL scored lower in aroma (4.48 ± 0.21^{ab}) and overall acceptability (3.92 ± 0.19^a), suggesting that the balance of flavors may not appeal to consumers as much as the more traditional options. These findings underscore the challenge of achieving desirable sensory characteristics when substituting or blending flours in bread formulations.³⁷ In contrast, the samples with higher percentages of wheat flour, such as the 10% CYF and 90% WF bread + FL, showed improved sensory attributes, indicating that retaining wheat flour in the formulation may enhance consumer acceptance. The evaluation of texture also highlights significant ($p < 0.05$) differences, with 100% WF bread achieving a texture score of 5.16 ± 0.22^c compared to the 50% CYF and 50% WF bread + FL, which received a much lower score of 3.34 ± 0.23^a . This suggests that the integration of cocoyam flour into the bread formulation may alter the expected texture, which is crucial for consumer enjoyment.³⁸ The lower textural scores for cocoyam-based products point to the need for further research on optimizing formulations to achieve a more palatable texture while maximizing the health benefits associated with cocoyam.

Table 2: Results of calorific value of Cocoyam/wheat flour composite Bread Sample in KJ/g

Sample	Calorific value (KJ/g)
COMMERCIAL BREAD	13.56 ± 0.13^g
100% WF BREAD	12.88 ± 0.17^f
100% CYF BREAD	8.56 ± 0.02^a
100% CYF BREAD + FL	8.96 ± 0.21^b
50% CYF + 50% WF BREAD + FL	8.54 ± 0.04^a
20% CYF + 80% WF BREAD + FL	10.31 ± 0.15^d
10% CYF + 90% WF BREAD + FL	11.29 ± 0.00^e
100% WF BREAD + FL	9.27 ± 0.01^c

Values are Mean plus or minus standard deviation (SD) with the same superscripts in the column are not significantly different ($p > 0.05$).

KEY:

WF = Wheat Flour
CYF = Cocoyam Flour
FL = Cocoa powder (Phenolic source)

Overall, the sensory evaluation indicates that while cocoyam-based breads can be nutritionally beneficial, achieving consumer acceptance may require careful formulation and blending strategies to enhance sensory characteristics. This aligns with research suggesting that sensory qualities are critical for market acceptance of novel food products.^{39, 40} Addressing these sensory challenges will be essential for promoting cocoyam-based products to health-conscious consumers seeking nutritious alternatives to traditional bread.

Proximate analysis of the composite bread.

Table 4 presents the detailed results of the proximate analysis conducted on the various formulations of composite bread as well as the control bread sample. The findings indicate that there were significant differences ($p < 0.05$) in several nutritional parameters when the values for the composite breads were compared with the control sample made entirely from wheat flour (100% WF bread). Notably, a significant increase was recorded in the values of moisture content particularly in the 100% CYF (cocoyam flour) bread along with increases in fat and crude protein contents of the composite bread samples.

The moisture content, expressed in percentage, demonstrated a distinct pattern among the bread formulations, reflecting the influence of cocoyam flour on water retention properties of the bread. The moisture content values followed this specific trend: 100% CYF bread (22.39 ± 0.07^g %) > 100% WF bread (18.15 ± 0.13^f %) > commercial bread (14.54 ± 0.06^e %) > 100% CYF bread + FL > 20% CYF + 80%

WF bread + FL (13.72 ± 0.09^d %) > 50% CYF + 50% WF bread + FL (12.01 ± 0.04^c %) > 10% CYF + 90% WF bread + FL (11.26 ± 0.06^b %) > 100% WF bread + FL (8.85 ± 0.08^a %). These values fall within the moisture content range previously reported for composite bread blends made from soy-cheese and wheat flour, as documented by

Odedeji et al. ⁴¹ Low moisture content is often desirable in wheat flour-based products, as it helps to enhance shelf life by inhibiting the growth of mould and minimizing chemical reactions that can degrade product quality. ⁴²

Table 3: Results of aroma, Taste, Texture, Colour and general acceptability of the composite bread

Samples	Aroma	Taste	Texture	Colour	General Acceptability
COMMERCIAL BREAD	6.76 ± 0.18^c	4.04 ± 0.17^{bc}	4.92 ± 0.15^d	5.20 ± 0.07^b	5.56 ± 0.22^d
100% WF BREAD	4.28 ± 0.25^a	4.36 ± 0.21^c	5.16 ± 0.22^c	6.48 ± 0.19^d	5.40 ± 0.22^{cd}
100% CYF BREAD	4.68 ± 0.18^b	3.84 ± 0.18^b	4.16 ± 0.17^b	4.56 ± 0.18^a	4.28 ± 0.17^b
100% CYF BREAD + FL	5.52 ± 0.27^d	4.08 ± 0.23^{bc}	4.20 ± 0.28^b	5.92 ± 0.23^c	4.56 ± 1.36^b
50% CYF + 50% WF BREAD + FL	4.48 ± 0.21^{ab}	3.36 ± 0.20^a	3.34 ± 0.23^a	4.76 ± 0.18^{ab}	3.92 ± 0.19^a
20% CYF + 80% WF BREAD + FL	5.00 ± 0.23^c	4.48 ± 0.25^c	4.44 ± 0.28^{bc}	5.32 ± 0.20^b	5.12 ± 0.22^c
10% CYF + 90% WF BREAD + FL	4.92 ± 0.26^c	4.48 ± 0.23^c	4.52 ± 0.25^c	5.64 ± 0.19^b	5.20 ± 0.18^c
100% WF BREAD + FL	4.71 ± 3.39^{bc}	4.63 ± 0.26^{cd}	4.38 ± 0.17^{bc}	5.63 ± 0.23^b	5.29 ± 0.18^c

Values are Mean plus or minus standard deviation (SD) with the same superscripts in the same column are not significantly different ($p > 0.05$).

KEY:

WF = Wheat Flour

CYF = Cocoyam Flour

FL = Cocoa powder (Phenolic source)

Table 4: Results of proximate composition of composite bread (%)

Samples	Moisture	Ash	Fat	Fibre	Crude Protein	CHO
COMMERCIAL BREAD	14.54 ± 0.06^c	0.50 ± 0.01^a	3.24 ± 0.04^a	1.80 ± 0.02^a	10.63 ± 0.16^b	$69.29^h \pm 0.02^h$
100% WF BREAD	18.15 ± 0.13^f	0.90 ± 0.01^b	4.07 ± 0.04^b	2.60 ± 0.01^b	14.12 ± 0.16^f	59.53 ± 0.03^a
100% CYF BREAD	22.39 ± 0.07^g	0.53 ± 0.03^a	3.66 ± 0.09^a	4.60 ± 0.01^c	8.38 ± 0.17^a	60.44 ± 0.09^b
100% CYF BREAD + FL	14.60 ± 0.12^c	0.83 ± 0.02^{ab}	6.24 ± 0.03^c	4.00 ± 0.07^{bc}	11.00 ± 0.06^c	63.33 ± 0.19^d
50% CYF + 50% WF BREAD + FL	12.01 ± 0.04^c	0.20 ± 0.06^a	7.46 ± 0.29^d	4.50 ± 0.01^c	14.50 ± 0.03^f	61.33 ± 0.03^c
20% CYF + 80% WF BREAD + FL	13.72 ± 0.09^d	0.90 ± 0.03^b	7.83 ± 0.07^d	3.60 ± 0.05^c	11.50 ± 0.09^c	63.26 ± 0.17^d
10% CYF + 90% WF BREAD + FL	11.26 ± 0.06^b	0.86 ± 0.03^b	4.56 ± 0.29^b	4.50 ± 0.04^c	13.25 ± 0.11^e	65.57 ± 0.13^f
100% WF BREAD + FL	8.85 ± 0.08^a	0.65 ± 0.02^{ab}	6.13 ± 0.29^c	3.00 ± 0.02^{bc}	12.75 ± 0.01^d	68.62 ± 0.12^g

CHO CONTENT = TOTAL CARBOHYDRATE

Values are Mean plus or minus standard deviation (SD) with the same superscripts in the same column are not significantly different ($p > 0.05$).

CHO = Total Carbohydrates.

KEY:

WF = Wheat Flour

CYF = Cocoyam Flour

FL = Cocoa powder (Phenolic source)

With regard to ash content, the results in Table 4 revealed no statistically significant difference ($p > 0.05$) among the various composite breads. Nevertheless, there was a slight increase in ash content with higher levels of wheat flour substitution by cocoyam flour. This reflects the mineral contribution of cocoyam to the bread, although the differences were not pronounced enough to be considered statistically significant.

The fat content of the composite bread samples also displayed an increasing trend in line with the increasing level of cocoyam flour substitution, with the notable exception of the 10% CYF + 90% WF bread + FL formulation. In contrast, the 20% CYF + 80% WF bread + FL sample recorded a higher fat content of 7.83 ± 0.07^d %, reflecting the combined lipid contribution of cocoyam and cocoa powder. This observation aligns with findings by Vossen et al. ⁴³ who reported that fat-congruent flavours can enhance the sensory perception of fat in food products, especially in protein-lipid blends such as drinks and

baked goods. Fibre analysis showed a significant ($p < 0.05$) increase in crude fibre content across the composite bread samples compared with the 100% WF control. The values clearly demonstrated a direct relationship between fibre content and the level of cocoyam flour inclusion. Specifically, fibre content ranged from the lowest value in the commercial bread sample (1.80 ± 0.02^a %) to the highest in the 100% CYF bread (4.60 ± 0.01^c %). This finding is consistent with the report by Ashaye et al. ⁴⁴ on biscuit formulations incorporating cassava and pigeon pea flours. Crude fibre plays a vital physiological role by promoting digestive health and binding dietary fats in the gastrointestinal tract. This binding capacity can help reduce the risk of lifestyle-related health conditions such as obesity, diabetes mellitus, and cardiovascular diseases. ⁴⁵

In terms of protein content, there was a statistically significant ($p < 0.05$) increase in the crude protein levels of the composite bread samples relative to the 100% WF control bread. The trend in protein content followed this descending order: 50% CYF + 50% WF bread +

FL (14.50 ± 0.03^f %) > 100% WF bread (14.12 ± 0.16^f %) > 10% CYF + 90% WF bread + FL (13.25 ± 0.11^e %) > 100% WF bread + FL (12.75 ± 0.01^d %) > 20% CYF + 80% WF bread + FL (11.50 ± 0.09^c %) > 100% CYF bread + FL (11.00 ± 0.06^c %) > commercial bread (10.63 ± 0.02^b %) > 100% CYF bread (8.38 ± 0.17^a %). The observed variations in protein content can be attributed to the naturally high protein level present in cocoyam, as well as the interactive effects of cocoa powder addition and flour ratio. The increasing protein levels in the composite breads, particularly those with a balanced proportion of CYF and WF, mirror the findings by Genga- Fabusiwa et al.⁴⁶ on biscuits made from pigeon pea-wheat composite flour, and by others who investigated millet-pigeon pea-based confectioneries.⁴⁷ Finally, the carbohydrate content of the bread samples varied inversely with protein and moisture contents, as expected. The carbohydrate percentage ranged from 100% CYF bread (59.29 ± 0.03^a %) to 100% WF bread + FL (68.62 ± 0.12^s %), with 100% WF bread recording the highest value at 69.29 ± 0.02^b %. These variations reflect the typical trade-offs in proximate composition as a result of ingredient substitution and the functional roles of individual components.

Table 5: Mineral Compositions of the composite bread (mg/100g)

Samples	K	Cu	Fe	Zn	Ca	Mg
COMMERCIAL BREAD	1.60 ± 0.04^a	2.10 ± 0.12^b	4.90 ± 0.18^a	0.67 ± 0.06^{ab}	1.40 ± 0.27^b	0.85 ± 0.61^b
100% WF BREAD	1.80 ± 0.05^a	1.40 ± 0.00^{ab}	5.30 ± 0.00^b	1.54 ± 0.06^c	1.44 ± 0.33^b	0.35 ± 0.90^a
100% CYF BREAD	5.81 ± 0.29^e	0.89 ± 0.06^a	5.90 ± 0.55^{bc}	0.78 ± 0.00^b	2.70 ± 0.56^c	0.82 ± 0.25^b
100% CYF BREAD + FL	5.97 ± 0.06^e	0.82 ± 0.00^a	5.02 ± 0.12^b	0.92 ± 0.12^b	1.56 ± 0.15^b	1.02 ± 0.09^c
50% CYF + 50% WF BREAD + FL	4.10 ± 0.15^d	1.15 ± 0.09^{ab}	7.47 ± 0.15^d	0.38 ± 0.09^a	4.16 ± 0.40^d	0.84 ± 0.05^b
20% CYF + 80% WF BREAD + FL	3.10 ± 0.35^c	1.50 ± 0.06^{ab}	6.21 ± 0.12^c	0.55 ± 0.01^a	0.74 ± 0.18^a	0.37 ± 0.28^a
10% CYF + 90% WF BREAD + FL	2.26 ± 0.67^b	2.30 ± 0.20^b	11.10 ± 0.23^c	0.91 ± 0.17^b	1.68 ± 0.64^b	0.71 ± 0.35^b
100% WF BREAD + FL	3.86 ± 0.07^d	0.93 ± 0.02^a	6.52 ± 0.58^c	0.39 ± 0.12^a	1.38 ± 0.16^b	0.52 ± 0.01^a

Values are Mean plus or minus standard deviation (SD) with carrying the same superscripts in the same column are not significantly different ($p > 0.05$).

KEY:

WF = Wheat Flour

CYF = Cocoyam Flour

FL = Cocoa powder (Phenolic source).

The trend observed in the copper (Cu) content among the bread samples is as follows: 10% CYF + 90% WF bread + FL (2.30 ± 0.20^b mg/100g) > commercial bread (2.10 ± 0.12^b mg/100g) > 20% CYF + 80% WF bread + FL (1.50 ± 0.06^{ab} mg/100g) > 100% WF bread (1.40 ± 0.00^{ab} mg/100g) > 50% CYF + 50% WF bread + FL (1.15 ± 0.06^{ab} mg/100g) > 100% WF bread + FL (0.93 ± 0.02^a mg/100g) > 100% CYF bread (0.89 ± 0.06^a mg/100g) > 100% CYF bread + FL (0.82 ± 0.00^a mg/100g). Regarding iron (Fe), the results show a significant increase ($p < 0.05$) in all the composite bread samples when compared to the control (100% WF bread). The trend of increasing Fe content is as follows: commercial bread (4.90 ± 0.18^a mg/100g) < 100% CYF bread + FL (5.02 ± 0.12^b mg/100g) < 100% WF bread (5.30 ± 0.00^b mg/100g) < 100% CYF bread (5.90 ± 0.55^{bc} mg/100g) < 20% CYF + 80% WF bread + FL (6.21 ± 0.12^c mg/100g) < 100% WF bread + FL (6.52 ± 0.58^c mg/100g) < 10% CYF + 90% WF bread + FL (11.10 ± 0.23^c mg/100g).

For zinc (Zn), there was a statistically significant decrease ($p < 0.05$) in the content across all composite bread samples compared to the control, which is the 100% WF bread (1.54 ± 0.06^c mg/100g). According to Wang et al.⁴⁸, enhancing zinc levels in wheat and its derived food products is a practical approach to combating zinc deficiency in humans, a condition linked to a range of health complications. Additionally, the calcium (Ca) content did not show a significant difference ($p > 0.05$) in most of the composite breads compared to the control, except for two formulations: 100% CYF

bread (2.70 ± 0.56^c mg/100g) and 50% CYF + 50% WF bread + FL (4.16 ± 0.40^d mg/100g), which were significantly higher than the control sample (1.40 ± 0.27^b mg/100g). Magnesium (Mg) content in the composite breads exhibited a significant increase ($p > 0.05$) compared to the control. The highest magnesium concentration was found in the 100% CYF bread + FL sample (1.02 ± 0.09^c mg/100g), while the 100% WF bread had the lowest value (0.35 ± 0.09^a mg/100g).

Determination of mineral contents of the composite bread

Table 5 presents the results of the mineral content analysis of the various composite breads, with values expressed in milligrams per 100 grams (mg/100g). The data clearly reveal that the composite breads formulated using blends of cocoyam flour (CYF) and wheat flour (WF) exhibit significant differences in the levels of certain macronutrients and micronutrients when compared to the control samples. Specifically, there was a statistically significant difference ($P < 0.05$) in the concentrations of macronutrients such as potassium (K) and calcium (Ca), whereas the magnesium (Mg) content did not show a significant variation. Furthermore, the composite breads demonstrated significantly higher levels of the micronutrient iron (Fe), while copper (Cu) and zinc (Zn) contents showed no significant difference ($p > 0.05$) across the samples.

bread (2.70 ± 0.56^c mg/100g) and 50% CYF + 50% WF bread + FL (4.16 ± 0.40^d mg/100g), which were significantly higher than the control sample (1.40 ± 0.27^b mg/100g). Magnesium (Mg) content in the composite breads exhibited a significant increase ($p > 0.05$) compared to the control. The highest magnesium concentration was found in the 100% CYF bread + FL sample (1.02 ± 0.09^c mg/100g), while the 100% WF bread had the lowest value (0.35 ± 0.09^a mg/100g).

The results for potassium (K), as shown in the table, revealed a marked and significant increase in all the cocoyam/wheat flour composite bread samples in comparison with the commercial bread, which served as the control. The order of increasing potassium concentration is as follows: 100% CYF bread + FL (5.92 ± 0.06^e mg/100g) > 100% CYF bread (5.81 ± 0.29^e mg/100g) > 50% CYF + 50% WF bread + FL (4.10 ± 0.15^d mg/100g) > 100% WF bread + FL (3.86 ± 0.07^d mg/100g) > 20% CYF + 80% WF bread + FL (3.10 ± 0.35^c mg/100g) > 10% CYF + 90% WF bread + FL (2.26 ± 0.67^b mg/100g) > 100% WF bread (1.80 ± 0.05^a mg/100g) > commercial bread (1.60 ± 0.04^a mg/100g). It has been reported that cocoyam-wheat flour breads are rich in potassium. Potassium plays a vital role in maintaining normal cellular function, enabling proper nerve transmission, and facilitating muscle contraction.⁴⁹ The increased potassium content in the composite breads could therefore enhance these physiological functions. The study further indicates that the

potassium content increased proportionally with the level of cocoyam flour supplementation in the bread formulations.

Moreover, it has been well-established that adequate dietary intake of potassium provides protective effects against a range of chronic health conditions such as high blood pressure, stroke, kidney failure, cardiac disorders, and osteoporosis, especially when potassium intake is balanced with a low sodium diet.^{50, 51}

Alkaloids, phytate and tannin contents of the composite bread.

Table 6 reveals the result of the phytochemical compounds screening of the cocoyam/wheat flour composite bread. It was revealed that these phytochemicals; Flavonoid, Alkaloid, Phytate, and Tannin are present in all the samples of bread except flavonoid that are absent in commercial bread, 100% WF bread and 100% CYF bread, however, Saponin, Glycoside, Phenol and Terpenoids were absent in all the samples of composite bread. Phytochemicals are naturally occurring compounds in plants that can provide protective health benefits to humans. While some phytochemicals also function as essential

Table 6: Qualitative screening of phytochemical compounds in cocoyam / wheat flour composite bread.

Name	Commercial bread	100% WF bread	100% CYF bread	100% CYF bread + FL	50% CYF + 50% WF bread + FL	20% CYF + 80% WF bread + FL	10% CYF + 90% WF bread + FL	100% WF bread + FL
FLAVONOID	-ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve
ALKALOID	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
SAPONIN	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
PHYTATE	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
GLYCOSIDE	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
TANIN	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
PHENOL	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
TERPENOID	--ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

KEY:

WF = Wheat Flour

CYF = Cocoyam Flour

FL = Cocoa powder (Phenolic source)

vitamins, many serve to protect the body's cells from oxidative damage caused by environmental toxins and the natural by-products of metabolic processes.⁵² Flavonoids, a prominent group of phytochemicals, are categorized based on their chemical structures. These compounds are widely found in medicinal herbs, which have traditionally been utilized for managing chronic conditions such as type-2 diabetes. Among the various phytochemicals, flavonoids and their derivatives have gained particular attention due to their potential hypoglycaemic effects.⁵³

The phytate content of the composite bread blends was evaluated and the results are shown in Table 7, expressed in milligrams per gram (mg/g). The analysis indicated that there was no significant difference ($p > 0.05$) in the phytate content of all the composite bread samples when compared to the control sample, which is the 100% wheat flour (WF) bread (3.76 ± 0.03^c mg/g), as well as the commercially available bread (1.71 ± 0.03^a mg/g). The trend observed in the phytate content across the composite bread formulations follows this sequence: 100% cocoyam flour (CYF) bread (3.79 ± 0.07^c mg/g) > 100% WF bread + FL (3.77 ± 0.02^c mg/g) > 20% CYF + 80% WF bread + FL (3.77 ± 0.03^c mg/g) > 10% CYF + 90% WF bread + FL (3.76 ± 0.01^c mg/g) > 100% WF bread (3.76 ± 0.03^c mg/g) > 100% CYF bread + FL (2.79 ± 0.02^b mg/g) > 50% CYF + 50% WF bread + FL (2.78 ± 0.03^b mg/g) > commercial bread (1.71 ± 0.03^a mg/g).

Phytate, also known as phytic acid, has been shown to exhibit anti-cancer properties.⁵⁴ It also acts as a natural antioxidant in foods, helping to prevent oxidative damage.⁵⁵ Furthermore, dietary phytate has been reported to reduce blood glucose levels by slowing down the rate of starch digestion and delaying gastric emptying, thereby modulating postprandial blood sugar spikes.⁵⁶ Although cocoyam is a nutritious food source, it contains certain antinutritional factors, including phytate, that may limit its utilization. However, it is noteworthy that such antinutrients can be significantly reduced through thermal processing techniques.⁵⁷ Consequently, evaluating

the phytate levels in cocoyam/wheat flour composite breads is not only important for determining their nutritional value but also crucial in understanding their potential health benefits. The findings from this study show that phytate content in the composite breads tends to increase with the proportion of wheat flour substitution. Additionally, the phytate values in these composite breads are generally lower when compared to those found in unfermented white cassava products,⁵⁸ and breads made from cocoa powder-flavoured, yellow-fleshed cassava/wheat flour composite blends.⁵⁹

The tannin content of the composite breads, also presented in Table 7 and expressed in mg/g, reveals a general decline in tannin levels when compared with the control sample of 100% WF bread. The order of tannin content among the various samples is as follows: commercial bread (3.14 ± 0.45^a mg/g) < 50% CYF + 50% WF bread + FL (3.61 ± 0.75^b mg/g) < 10% CYF + 90% WF bread + FL (3.68 ± 0.62^b mg/g) < 100% CYF bread (3.91 ± 0.16^c mg/g) < 100% WF bread + FL (3.93 ± 0.34^c mg/g) < 100% CYF bread + FL (4.21 ± 1.60^d mg/g) < 20% CYF + 80% WF bread + FL (4.29 ± 1.20^d mg/g) < 100% WF bread (4.67 ± 0.42^e mg/g).

Tannins are known to possess various bioactive properties, including antiviral,⁶⁰ antibacterial,⁶¹ and antiparasitic activities.⁶² Despite these benefits, the levels of tannin observed in the composite breads were generally lower compared to those found in bread products made from cocoa powder-flavoured, yellow-fleshed cassava/wheat flour composite flours.⁵⁹ In addition, the total alkaloid content, measured in mg/g and presented in Table 7, showed a statistically significant ($p < 0.05$) increase in certain composite bread samples. Specifically, 100% CYF bread + FL (8.20 ± 0.20^d mg/g) and 50% CYF + 50% WF bread + FL (8.03 ± 0.34^d mg/g) had significantly higher alkaloid contents when compared with the control sample, 100% WF bread (2.46 ± 0.42^a mg/g). The trend of increasing alkaloid content across the bread samples is as follows: 100% CYF bread + FL (8.20 ± 0.20^d mg/g) > 50% CYF + 50% WF bread + FL (8.03 ± 0.34^d mg/g) > commercial bread (4.32 ± 0.63^c mg/g) > 20% CYF + 80% WF bread +

FL (4.29 ± 0.67^c mg/g) > 10% CYF + 90% WF bread + FL (4.05 ± 0.15^c mg/g) > 100% WF bread + FL (3.19 ± 0.51^b mg/g) > 100% CYF bread (2.98 ± 0.17^{ab} mg/g) > 100% WF bread (2.46 ± 0.42^a mg/g).

Table 7: Results of Phytochemical analysis (mg/g).

Sample	(Phytate)	(Tannin)	(Alkaloids)
COMMERCIAL BREAD	1.71 ± 0.03^a	3.14	4.32 ± 0.63^c
100% WF BREAD	3.76 ± 0.03^c	4.67	2.46 ± 0.42^a
100% CYF BREAD	3.79 ± 0.07^c	3.91	2.98 ± 0.17^{ab}
100% CYF BREAD + FL	2.79 ± 0.02^b	4.21	8.20 ± 0.20^d
50% CYF + 50% WF BREAD + FL	2.78 ± 0.03^b	3.61	8.03 ± 0.34^d
20% CYF + 80% WF BREAD + FL	3.77 ± 0.03^c	4.29	4.29 ± 0.67^c
10% CYF + 90% WF BREAD + FL	3.76 ± 0.01^c	3.68	4.05 ± 0.15^c
100% WF BREAD + FL	3.77 ± 0.02^c	3.93	3.19 ± 0.51^b

Values are Mean plus or minus standard deviation (SD) with the same superscripts in the same column are not significantly different ($p > 0.05$).

KEY:

WF = Wheat Flour

CYF = Cocoyam Flour

FL = Cocoa powder (Phenolic source)

Alkaloids are known for their wide-ranging pharmacological effects. In the context of cocoyam/wheat flour composite breads, these compounds may contribute to potential anti-inflammatory and antimicrobial benefits. Scientific studies have shown that specific alkaloids can modulate immune responses and offer protection against microbial pathogens.⁶³ Plants, in general, serve as a rich reservoir of bioactive compounds with numerous therapeutic potentials. These include antiviral, anticancer, analgesic, antitubercular,⁶⁴ antiproliferative, antibacterial, and antioxidant properties, which could be harnessed for drug development and health promotion.⁶⁵

Conclusion

The data obtained from this study clearly demonstrated that the cocoa powder-flavored composite bread, developed through the supplementation of wheat flour (WF) with cocoyam flour (CYF), has the potential to serve as a cost-effective and health-promoting dietary option. The findings suggest that this composite bread formulation could offer a safe, affordable, and nutritionally advantageous alternative to conventional bread, especially in regions with limited access to wheat-based products.

Remarkably, the complete substitution of wheat flour with cocoyam flour, coupled with the fortification using cocoa powder; represented by the 100% CYF + FL formulation led to a significant improvement in the overall nutritional profile of the bread. This enhancement was marked by increased levels of essential nutrients, including protein, crude fiber, ash content, and iron (Fe). In addition to these nutritional

gains, the formulation exhibited notable antioxidant properties, attributed to the phytochemical constituents naturally present in both cocoyam and cocoa powder. Furthermore, the inclusion of cocoa powder in the composite bread did not only impart a pleasant flavor and aroma, which contributed to the product's sensory appeal, but also played a vital role in boosting its nutritional composition. The cocoa powder served as an additional source of phenolic compounds, thereby improving the antioxidant capacity and contributing to the functional quality of the final product. Despite these worthwhile research findings, the study acknowledges the need for further investigation to substantiate these outcomes. Specifically, controlled animal trials are recommended as a follow-up study to validate the nutritional and health benefits observed, particularly with regard to bioavailability, metabolic impact, and potential long-term health implications of regular consumption of the composite bread.

Conflict of Interest

The authors declare no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors wish to express their profound appreciation to the Department of Biochemistry laboratory unit of Ladoké Akintola University of Technology, Ogbomosho, Nigerian Institute of science Laboratory Technology multipurpose laboratory, Samonda, Ibadan and the Hegada scientific laboratory, Sango, Ibadan for given us the opportunity to carry out the research work in their laboratories.

References

- Si H, Zhang N, Tang X, Yang J, Wen Y, Wang L, Zhong X. Transgenic research in tuber and root crops. *Genetic Engin Horticult crop*. 2018; 225-248.
- Otekin OA, Sawiaka B, Adeyinu AG, Otekin OA. Cocoyam (*Colocassia esculenta* [CL]) Schott: Exploring the production, health and trade potential in sub-saharan African. *Sustainability* 2021; 13: 4483.
- Nkeme KK, Ekanem JT, Nse VA. Capacity building needs of small-holder cocoyam (*X. sagittifolium*) farmer in selected rural communities of Akwa Ibom State, Nigeria. *J Agric Exten*. 2021; 25(2): 32-42.
- RBT Newsletter. Cocoyam has huge market but few farmers cultivate its. *Roots, Tubers and Bananas Newsletter*; 2020a.
- FOA. The future of livestock in Nigeria. Opportunity and challenges in face of uncertainty, Rome, Italy 2019.
- Rashmi DR, Raghu N, Gopenath TS, Pradeep P, Pugazhandi B, Murugesan K, Ashok G, Ranjith MS, Chandrashekrappa GK, Kanthesh MS. Taro (*Colocassia esculenta*): An overview. *J Med Plants Studies*; 2018; 6 (4): 156-161.
- Ojinnaka MC, Nnorom CC. Quality evaluation of Wheat-Cocoyam-Soyabean cookies. *Nigerian J Agric, Food and Environ*. 2015; 11 (3): 123-129.
- Wodemariyam MM, Gebre A, Neme GP Effects of boiling, soaking and fermentation on nutritional, antinutritional and functional properties of Tannia cocoyam (*Xanthosoma sagittifolium*) corn flour and (*Doctoral dissertation*). (Ph.D thesis, Dept., Unvi., Conutry). 2022.
- Kabuo NO, Alagbaoso OS, Omeire GC, Peter-Ikechukwu AI, Akajiaku LO, Obasi AC. Production and Evaluation of Biscuit from Cocoyam (*Xanthosoma sagittifolium* Cv

- Okoriko)- Wheat Composition Flour. Res J Food Nutr. 2018; 2 (2): 53-61
10. Sanful RE, Darko S. Production of cocoyam, cassava, and wheat flour composite rock cake. Pakistan J Nutr. 2010; 9(8), 810-814. ISSN 1680-5194.
 11. Mba CJ, Agu HO. Developments on the bioactive compounds and food uses of the tubers: *Colocasia esculenta* (L) Schott (Tannia). Asia J Food Sci. 2022; 20 (11): 101-112.
 12. Bradbury JH, Holloway WD. Antinutritional factors in root crops. The chemical composition of tropical root crops; significance for nutritional and agriculture in the pacific 1988; (Cap. 5. pp. 101-119). Canberra: ACIAR.
 13. Onwueme IC, Charles WB. Utilization, socio-economic perspective and future prospects. In IC. Onwueme, and WB. Charles (Eds.), Tropical root and tuber crops; production, perspectives and future prospects 1994; (chap. 20, pp. 156-158, FAO Plant Production and Protection Papers, No.126). Rome: FAO.
 14. Owusu-Darko PG, Paterson A. Omenyo EL. (2014). Cocoyam (corms and cormels) an underexploited food and feed resource. J Agric Chem Environ. 2014; 3(01): 22-29. <http://dx.doi.org/10.4236/jacen.2014.31004>.
 15. Ahmed I, Lockhart PJ, Agoo EM, Naing KW, Nguyen DV, Medhi DK, Matthews PJ. Evolutionary origin of taro (*Colocasia esculenta*) in Southeast Asia. Ecol. Evol. 2020; 10, 1-14.
 16. Okoli IC. Cocoyam as animal feed 1: Production dynamics and nutrients composition. 2020; <https://researchtropica.com/cocoyam-as-anima-feed-1/>
 17. Brown AC, Ibrahim SA, Song D. Poi's history, uses and roe in health. In: Fruits, vegetables and herbs. 2016, Elsevier B. V., Amsterdam, The Netherlands.
 18. Bhagyashree RP, Hussein MA. Anthepatexic effects of *Colocasia esculenta* leaf juice. Int. J Adv Biotech Res. 2011; 2: 296-304.
 19. Ogunmefun OT. God's endowment of curative power in plant. <https://dx.doi.org/10.57772/intecopen.2018; 77423>.
 20. Kokoh AAM, Konan BN, Gnoumou JIK, Elleingand E, Koffi E. Effect of incorporation of yam flour and moringa powder in wheat bread on glycemic response. Food Nutr Sci; 2022; 13 (99): 781-796.
 21. Abass AB, Awoyale W, Aklenkhe B, Malu N, Asiru BW, Manyong V, Shangina N. Can food technology innovation change the status of a food security crop? A review of cassava transformation into "bread" in Africa. Food Int; 2018; 34 (1): 87-102.
 22. Ishaya FA, Oshodi AA. The proximate composition and sensory evaluation of the flours of breadfruit (*Artocarpus altilis*), Benth seed (*Adenopus breviflorus*) and their composite bread. Chem Materials Res; 2013; 3(9): 73-84.
 23. Oluwajoba SO, Malomo O, Ogunmoyela OAB, Dudu OEO, Odeyemi A. Microbiological and nutritonal quality of warnakashi enriched bread. J Micro, Biotech Food Sci. 2012; 2(1): 42-68.
 24. Olaoye OA, Onilude AA, Oladoye CO. "Breadfruit flour in biscuit making: effects on product quality," Afr J Food Sci. 2007; 1, 20-23.
 25. AOAC. Official methods of analysis of the Association of Official Analytical chemists (15th ed.). 2005; Arlington, VA: Author.
 26. Potter NN. Hedonic Scale: Food science; Westport, C.T: The AVI Publishing Company Inc. 1968
 27. Deliza R, Macfic HJH. The generation of sensory expectation by external by external cues and its effecting sensory perception and hedonic ratings. J Sensory stud; 1996; 11: 103-28.
 28. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lighfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive compounds from plant Extracts. Plants; 2017; 6 (4):42. Doi: 10.3390/plants6040042.
 29. Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some iraian medicinal plants. 2008; Thai J. Pharm Sci, 32:17-20.
 30. Medoua GN, Mbome IL, Agbor-Egbe T, Mbofung A. Influence of fermentation on some quality characteristics of trifoliate yam (*Dioscorea dumetorum*) hardened tubers. Food Chemistry; 2007; 107 (3): 1180-1186.
 31. Wheeler EL, Ferrel RE. A method of phytic acid determination in wheat fractions. Cereal chem.; 1974; 48:312-316.
 32. Zar JH. Biostatistical Analysis, 1984; Prentice-Hall, Inc., USA, 620P.
 33. Biomass Energy Data Book Energy efficiency and Renewable Energy, 4th Edition. 2011; U.S Department of Energy.
 34. Abel ES, Ejiroghene RE, Ogechi TC. Evaluating the proximate chemical composition and sensory properties of composite bread from wheat and cocoyam flours. Afr J Food Sci. 2023; 17(5) pp. 74-84
 35. Ogunniyi LT, Ekechukwu AA. Nutritional and antinutritional evaluation of cocoyam (*Xanthosoma sagittifolium*) flour. Food Sci Nutr. 2023; 11(2), 112120.
 36. Adesina TJ, Salami AT, Okolie JU, Olaleye KD. Cocoyam flour: A potential functional food ingredient. Intern J Food Sci. 2023; 6(1), 45-54.
 37. Njintang Ngwene Y, Mbofung Collins MF, Facho B. Production, physicochemical, and sensory properties of cocoyam-based products. J Food Sci. Techn. 2021; 58(4), 346-358.
 38. Kinsella JE, Singh B. "The role of dietary fiber in human health: A review. "Crit Reviews Food Sci Nutr. 2020; 60 (16), 2774-2794.
 39. Ezeonu IM, Okeke OC, Onwuka GI, Agbo CI. Antioxidant activity and nutritional composition of cocoyam-wheat blends. Afr J Food Sci. 2021; 14(2), 23-29.
 40. Udom IM, Adedoyin ST, Olufemi AI. The impact of cocoyam cultivation on rural economic development in Nigeria. J Rural Stud. 2023; 90, 116-125.
 41. Odedeji JO, Ojo A, Arogundade LA, Oyeleke GO. Proximate composition and consumer's acceptability of bread produced from blends of soy-cheese and wheat flour. J Environ sci Toxicol Food Techn. 2014; 8(2), 41-44.
 42. Sigh A, Hung Y, Corredig M, Philips RD, Chinnan MS, McWatters KS. "Effect of milling method on selected physical and functional properties of cowpea (*Vigna Unguiculata*) paste" Intern J Food sci Techn; 2005; 1 (40): 525-536.
 43. Vossen RM, Gelin JL, Hettinga K, Andrew R, Erni P, Badnar I, Cayeux I. Improving plant-based protein drinks: The potential of flavors for cross-modal enhancement of fat perception. LWT-Food Sci Techn. 2024; 213. <https://doi.org/10.1016/j.lwt.2024.117016>.
 44. Ashaye OA, Olanipekun OA, Ojo SO. Chemical and nutritional evaluation of biscuits processed from cassava and pigeon pea flour. J Food Process. Techn. 2015; 6, 521. Available online: www.rtb.cgiar.org (accessed on 5 February 2021).
 45. American Diabetes Association. Diagnosis and classification of diabetes mellitus (published correction

- appears in *Diabetes Care*, 2010; 33 (4): e57]. *Diabetes care*; 33(Suppl. 1), S62-S69. <https://doi.org/10.2337/dc10-S062>
46. Gbenga-Fabusiwa FJ, Oladele EB, Oboh G, Adefegha SA. Nutritional properties, sensory qualities and glycemic response of biscuit produced from pigeon pea-wheat composite flour. *J Food Biochem*. 2018; [wileyonlinelibrary.com/journal/fbc](https://onlinelibrary.wiley.com/journal/fbc).
 47. Omah EC, Okafor GI. Production and quality evaluation of cookies from blends of millet-pigeon pea composite flour and cassava coetex. *J food Resource Sci*. 2015; 4, 29-31.
 48. Wang M, Kong F, Liu R, Fan Q, Zhang X. Zinc in wheat Grain, processing, and Food. *Front Nutrition*; 2020; 18 (7) 124. doi:10.3389/fnut. 2020.00124.
 49. Orhevba BA, Ndanaimi Y. Proximate and sensory properties of wheat-cocoyam (*Colocasia esculenta*) associated with the prevalence of type-2 diabetes mellitus in Chinese adults. *European J Nutr*; 2019; 58 (2): 819-830.
 54. Pires SMG, Reis RS, Cardoso SM, Pezzani R, Paredes-Osses E, Seilkhan A, Ydyrys A, Martrell M, Gurere ES, Setzer WN, Razis AFA, Modu B, Calina D, Sharifi-Rad J. Phytates as a natural source of health promotion: A critical evaluation of clinical trials. *Front. Chem*; 2023; 11:1174109. Doi: 10:3389/fchem:2023.1174109.
 55. Abdulwaliyu I, Arekemase SO, Adudu JA, Batari ML, Egbule MN, Okoduwa SIR, Investigation of the medicinal significance of phytic acid as an indispensable anti-nutrient in disease. *Clin. Nutr. Exp*. 2019; 8, 42-61.
 56. Bloot APM, Kalschnne DL, Amaral JAS, Baraldi IJ, Canan C. A review of phytic acid sources, obtention, and applications. *Food Review Int*. 2021; 39, 73-92.
 57. Bello FA, Oladeji BS Physicochemical and Antioxidant properties of cocoyam, African Yam Bean, Watermelon rind Flour blends and consumer of acceptability its cake. *IPS J. Nutr Food Sci*; 2024; 3 (3): 214-221.
 58. Vilanculos SL, Svanberg U, Andlid T. Phytate degradation in composite wheat/cassava/sorghum bread: Effect of phytate-screening yeasts and addition of yeast extracts. *Food Sci Nutr*. 2023; 12(1): 216-226.
 59. Ajani RA, Oboh G, Adefegha SA, Akindahunsi AA, Nwokocha KE. Sensory attributes, Nutritional qualities and glycemic indices of bread blend produced from cocoa powdered flavored yellow fleshed cassava-wheat composite flours. *J Food Proc Preserv*. 2020; 44(9): e14673.
 - composite bread. *European J Agric Food Sci*. 2021; 3(3), 1057-1057. <https://doi.org/10.24018/ejfood.2021.3.3.297>
 50. World Health Organization. Increasing potassium intake to reduce blood pressure and risk of cardiovascular disease in adults. 2023; Retrieved 9 Aug.
 51. Aburto NJ, Hanco S, Gutierrez H, Hooper L, Elliott P, Cappuccio FP. Effect of increasing potassium intake on cardiovascular risk factors and disease: systematic review and meta-analysis. *British Med J*; 2013; 346: f1373.
 52. Ogunmefun OT. God's endowment of curative power in plant. <https://dx.doi.org/10.57772/intecopen>. 2018; 77423.
 53. Yao Z, Gu Y, Zhang Q, Liu L, Meng G, Wu H, Xia Y, Bao X, Shi H, Sun S, Wang X, Zhou M, Jia Q, Wu Y, Song K, Gao W, Guo C, Niu K. Estimated daily quercetin intake and
 60. Haung Q, Liu X, Zhao G, Hu T, Wang Y. Potential and challenges of tannin as an alternative to in-feed antibiotics for farm animal production. *Animal Nutr*. 2017; 4 (2): 137-150. Doi: 10.1016/j. aninu.2017.09.004.
 61. Gomathy G, Vijay T, Sarumathy K, Gunasekaran S, Palani S. Phytochemical screening and GC-MS analysis of *Mukia maderaspatana* (L.) leaves. *J Appl Pharm Sci*. 2012; 2 (12): 104-106.
 62. Baniel JMC. Phytochemical analysis and antioxidant activity evaluation of ethanolic of pitcher plant pitchers (*Nepenthes bellii*). *Int. J Biosci*. 2023; 22 (2): 66-72.
 63. Faisal S, Badshah SL, Kubra B, Emwas A, Jaremko M. Alkaloids as a potential antiviral: A comprehensive review; Natural products Bioprospecting; 2023; 13:4. <https://doi.org/10.1007/s13659-022-00366-9>.
 64. Zandavar H, Babazad MA. Secondary metabolites: Alkaloids and Flavonoids in medicinal Plants. *Herbs and species-New Adv*. 2023; 108030. Doi:10.5772/intechopen.108030.
 65. Qui S, Sun H, Zhang AH, Xu HY, Yan GL, Han Y, Wang XJ. Natural alkaloids: basic aspects, biological roles, and future perspectives. *Chinese J Nat Med*. 2014; 12: 0401-0406.