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Assessment of cocoyam, red kidney bean, and mango-based complementary foods: Influenced of fermentation and malting on quality attributes

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Abstract
Aim: To assess the influence of fermentation and malting on the pH, TTA, Vitamins, Phytochemical, antioxidant and Microbial quality of cocoyam, red kidney beans and mango - based complementary foods.
Methodology: Flour prepared from cocoyam, red kidney beans and mango was blended as follows: Fermented cocoyam/ malted red kidney beans/ mango; Fermented cocoyam/ non- malted red kidney beans/ mango; Non- fermented cocoyam/ malted red kidney beans/ mango; Non- fermented cocoyam/ Non- malted red kidney beans/ mango. The ratio was arrived at, through material balancing to give 16 g protein/100 g. samples were subjected to pH, TTA, Vitamins, Phytochemical, and antioxidant and Microbial analysis.
Results: Fermentation and malting decrease the pH and increase the TTA. pH varies from 6.57±0.03 (0 h) - 3.99±0.01 (96 h) and 6.81±0.03 (0 h) - 6.69±0.14 (72 h); TTA ranged from 0.12±0.04 (0 h) - 3.36±0.02 (96 h) and 0.16±0.06 (0 h) - 0.96±0.02 (72 h) during fermentation and malting respectively. Vitamins, Phytochemical, antioxidant and Microbial properties of the complementary foods increased significantly ($p< 0.05$) with fermentation and malting; β-carotene (30.04±0.25 - 18.55±0.25 µg /100 g), vitamin C (7.43±0.02 -9.85±0.02 mg/100 g). Total phenolic (2.16 ±0.43 - 4.23±0.03 mgGAE/100 g), DPPH (41.74±0.13 - 31.46±0.04 µmol TE/100 g), FRAP (6.75±0.04 - 14.26±0.02 mgAAE/100 g). Microbial load of the products was within acceptable limit.
Conclusion: Fermentation and malting improved the vitamin, phytochemical, and microbial quality of the complementary foods.

Keywords: Fermentation**,** malting**,** vitamins**,** phytochemical**,** antioxidant**,** microbial

Introduction
In recent years, there has been steady progress in the quest to replace expensive complementary food produced with sophisticated technology with those produced with low-cost technology and readily available cheap raw materials. The term complementary food can be defined as either solid or liquid substances containing nutrients other than breast milk that is given to young children/infants aged 6 to 24 months (Ali *et al*., 2021) [8]. As a result, complementary food has become necessary because an infant's growth in the first two years is extremely rapid, and breastfeeding alone cannot meet the child's nutritional needs. According to Skau *et al*. (2015) [52], DiMaggio *et al*. (2017), the ability of breast milk to meet the requirements for macronutrients and micronutrients becomes limited as infants' ages increase. However, the capacity of a complementary diet to meet the micronutrients requirement of infants depends on its nutritional quality. It is well known that the high cost of fortified complementary foods in many parts of developing countries is beyond the reach of most families (Muhimbula *et al*., 2011) [38], hence many families depend on inadequately processed and low quality traditional complementary. That is why micronutrients - malnutrition is among the major infant problem in developing countries Therefore, poor quality complementary food is a major cause for the high incidence of child malnutrition, morbidity, and mortality in many developing countries (Ali *et al*., 2021) [8].

To reduce these problems, low cost indigenous and unexploited legumes which can be processed and when properly complemented with commonly available carbohydrate sources will provide relatively affordable complementary foods that will help to alleviate micronutrients malnutrition and improve infants' nutrition (Amankwah *et al*., 2009) [10]. In developing countries cocoyam, red kidney bean and mango flour if properly processed and formulated complement breast milk as complementary foods, because a well-formulated and high nutrient food is necessary if complementary foods or foods other than breast milk are to be given to infants.

Fermentation and malting are simple technologies that can be adopted at the household level to enhance the nutritional value of local foods. Fermentation, a process involving the biochemical activity of beneficial microorganisms, can increase the bioavailability of nutrients, reduce anti-nutritional factors such as phytates and tannins, and introduce beneficial probiotics. For example, fermented foods often have higher levels of B-vitamins and improved protein digestibility (Adeyeye *et al*., 2020) [6]. Fermentation can lead to the breakdown of complex phytochemicals into more bioactive or bioavailable forms. For example, fermentation of legumes and cereals often increases

isoflavone aglycones, which have higher antioxidant activity (Dey & Kuhad, 2014) [16]. Fermentation can enhance antioxidant activity by producing peptides and polyphenol metabolites with strong radical scavenging properties (Hur *et al*., 2014) [28]. Additionally, microbial activity during fermentation may reduce oxidative stress markers in food systems.

Malting**,** which involves soaking, germinating, and drying grains, similarly enhances nutritional quality by activating enzymes that break down complex compounds (Dodo *et al*., 2018 [19]; Nkhata *et al*., 2018) [42]. This process reduces starch content while increasing free amino acids and certain vitamins, particularly vitamin C and some B-vitamins. Malting also helps in reducing anti - nutrients and improving mineral bioavailability, making it particularly valuable in the preparation of weaning foods and traditional beverages. Both fermentation and malting are low-cost, require minimal equipment, and can be implemented with locally available materials, making them especially suitable for resource-limited settings seed (Dodo *et al*., 2018; Belcar *et al*., 2021) [13, 19]. Their integration into household food practices can contribute significantly to improving dietary diversity, food safety, and nutritional outcomes, particularly among vulnerable populations such as children. This research work is aimed at determining the influence of malting and fermentation on the on the pH, TTA, Vitamins, Phytochemical, antioxidant and Microbial quality of cocoyam, red kidney beans and mango - based complementary foods.

Materials and Methods

1. Preparation of Raw Materials

Preparation of non- fermented cocoyam flour

Cocoyam was washed with water to eliminate soil particles and dirt. The corm peel and thick skin layer (corm pulp outer layer of approximately 5 mm thickness) was removed and the pulp was sliced (1cm thickness). The slices were washed, blanched in potable water at 85 ºC for 5 minutes using a gas cooker. The cooked slices were dried at 55 °C for 24 hours in dehydrators. Dried sample was ground, sieved (0.5 mm sieve) and packaged in low density polyethylene bags (Coronell-tovar *et al*., 2019) [15].

Preparation of fermented cocoyam flour

Cocoyam was washed with water to eliminate soil particles and dirtiness. The corm peel and thick skin layer (corm pulp outer layer of approximately 5 mm thickness) was removed and the pulp was sliced (1cm thickness). The slices were washed, blanched in potable water at 85 ºc for 5 minutes using a gas cooker. The cooked slices were dried at 55 °C for 15 hours in dehydrators. Dried sample was ground, sieved (0.5 mm sieve) and packaged in low density polyethylene bags (Coronell-tovar *et al*., 2019) [15]. One hundred and twenty grams (120 g) of flour was mixed with 80 mL of distilled water in a 500 mL beaker which was covered and the slurry allowed to undergo natural fermentation at room temperature (30 ± 2 ºC). Fermentation was slurry by adding 50 % fermenting (back-slopping) slurry to fresh concentrate at 24 hours intervals over a period of 96 h when the pH stabilized. The fermented slurry were dehydrated in an air draft dehydrator at 50 ºC for 12 h to obtain fermented cocoyam flour (Ariahu *et al*., 1999) [11].

Preparation of non-malted red kidney beans flour

The kidney beans flour was prepared using the method described by Inyang *et al*. (2018); Ukeyima *et al*. (2019); Noah and Adedeji, (2020); Forwoukeh *et al*. (2023) [1, 27, 29, 56]. The bean was thoroughly cleaned. This was followed by soaking in clean water for 12 hours and the water was changed every 6 hours to prevent fermentation. After this the beans were washed twice with clean water, cooked in hot water at 85 ºC for 30 minutes, and washed with fresh water. The blanched bean was dried in a dehydrator at 55 ºC for 20 hours and dehulled by hand rubbing. The dried bean was milled into flour, sieved (0.5 mm) and stored in air-tight containers.

Preparation of malted red kidney beans flour

The malted red kidney beans flour was prepared according to the method of Okoye *et al*. (2021) [46]. Exactly 1 kg of red kidney beans seeds which was free from dirt and other extraneous materials was thoroughly cleaned and steeped in 2.5 litres of potable water in a plastic bowl at room temperature (30±2 ºC) for 12 hours with a change of water at intervals of 6 hours to prevent fermentation. The seeds were then rinsed for five consecutive times with excess water and cast on a moistened jute bag, covered with a polyethylene bag and left for 24 hours to fasten sprouting. The seeds were spread on the jute bag and allowed to germinate at room temperature (30±2 ºC) for 72 hours until the rootless reached a length of 1.5 cm. During this period, the seeds were sprinkled with water at intervals of 6 hours to facilitate germination. Non-germinated seeds were handpicked and discarded and the germinated seeds was cooked (85 ºC, 30 minuts), spread in a dehydrator at 55 ºC for 20 hours. The dried malted red kidney beans seeds were cleaned and rubbed in-between palms to remove the roots and shoots along with the hulls. The dehulled malted seeds was milled in attrition mill and sieved through a 0.5 mn mesh sieve. The flour produced was packaged in an airtight container.

Preparation of mango flour

Ripe mature mangoes (*Mangifera indica 'Alphonso'*) popularly known as Brockley was washed manually using potable water. The mango was peeled using a stainless steel knife and the pulp sliced using a manual food slicer in to smaller sizes of approximately 1cm thickness to facilitate the drying process. The sliced mango pulp was blanched for 5 mins at 70 ºC and dried using a dehydrator at 55 ºC for 24 hours to obtain mango flakes. The mango flakes were ground using laboratory grinders (M/S Sujata: New Delhi India) to obtain the flour. The mango flour was sieved through a 0.5 mm size mesh (Izidoro *et al*., 2023) [30].

2. Product formulation

Flours from cocoyam, red kidney beans and mango fruits was blended in different proportions or ratio as shown in Table 1. This ratio was arrived at, based on their protein content through material balancing to give 16 g protein/100 g food as recommended by world food programme (WFP) 2018, for infant diets (complementary foods). From the treatment combinations, four samples (FMM, FNMM, NFMM, NFNMM,) were generated with the aid of the material balancing. Nestle CERALAC, a commercial cereal based weaning food was used as control (CS).

Table 1: Complementary foods formulation Ingredients mix (g/100) by materials balancing

Complementary foods	Non- fermented cocoyam flour (g)	Fermented cocoyam flour (g)	Non-malted red kidney beans (g)	Malted red kidney beans (g)	Mango flour (g)
FMM	-	37.83	-	52.17	10
FNMM	-	24.32	65.68	-	10
NFMM	31.19	-	-	58.81	10
NFNMM	19.18	-	70.82	-	10

FMM: Fermented cocoyam/ malted red kidney beans/ mango
FNMM: Fermented cocoyam/ non- malted red kidney beans/ mango
NFMM: Non- fermented cocoyam/ malted red kidney beans/ mango
NFNMM: Non- fermented cocoyam/ Non- malted red kidney beans/ mango

3. Determination of pH

Determination of pH was done by the method of Onyango *et al*. (2013) [48]. The pH meter (TOA pH Meter HM–7B, Tokyo, Japan) was standardized using buffer solutions of acidic and basic values of 4.01 and 9.08 at 25 °C. The electrode was rinsed with distilled water before taking measurements. The fermented samples, (slurry mixtures of flour and water) were homogenized by stirring to achieve uniformity. pH readings were taken by dipping the electrode in the fermented mix and measurements taken from the display screen when the readings stabilized. Malted red kidney beans were milled (wet milling) prior to pH determination and following the same procedure as outlined above, the pH was measured.

4. Total titratable acidity (TTA)

The TTA analysis was done using AOAC method as described Onyango *et al*. (2013) [48]. Approximately 10 mL of sample was pipetted into a conical flask and two drops of phenolphthalein indicator used. Titration was done using 0.1N NaOH to a faint pink colour for at least one min (compared against a white background). The titre volume was noted and used for calculations of TTA which was expressed as percentage lactic acid. Calculations of TTA was determined and expressed as follows:

$$\%Lactic\ acid = \frac{Ax0.009x100}{V}$$

Where: A = mL of 0.1 NaOH required for the titration; and V = mL of sample taken for the test. 0.009 is a Constant.
Malted red kidney beans was milled (wet milling) prior to TTA determination and following the same procedure as outlined above TTA was measured.

5. Determination of the Vitamin Content of Complementary Foods

β carotene (Vitamin A) determination

Vitamin A was determined by the calorimetric method of Kirk and Sawyer (2015). Approximately 1 g of the sample and standard was mixed with 30 mL of absolute alcohol and 3 mL of 5 % KOH solution is added to it and is boiled for 30 min under reflux. After washing with distilled water, vitamin A is extracted with 150 mL of diethyl ether. The extract was evaporated to dryness at low temperature and then dissolved in 10 mL of isopropyl alcohol. Exactly 1 mL of standard vitamin A solutionwas prepared and that of the dissolved extract was transferred to separate cuvettes and their respective absorbance were read in a spectrophotometer at 325 nm with a reagent blank at zero.

$$Conc.of\ vit\ A\ in\ sample = \frac{Abs\ of\ sample}{Abs\ of\ std}\ X\ conc.of\ STD$$

Determination of thiamine (B_1) content

The spectrophotometric method, described by Wekhe and Chuku, (2021) [58] was used for determination of the vitamins B1. Exactly 5 g of each sample was homogenized with 50 mL of 1M ethanolic sodium hydroxide and the homogenate was filtered to obtain the filtrate used for the analysis. An aliquot (10 mL) of the filtrate was treated with equal volume of 0.1 M $K_2Cr_2O_7$ (potassium dichromate) solution in a flask. Standard thiamine solution was prepared and diluted to a chosen concentration (0.5). An aliquot of the standard thiamine solution was also treated with 10 mL of the dichromate solution ($K_2Cr_2O_7$) in a separate flask while a reagent blank was set up by treating l0 mL of the ethanolic sodium hydroxide with the potassium dichromate solution. The absorbance of the sample and the standard solutions was measured in a spectrophotometer at a wavelength of 360 nm with the reagent blank used to calibrate the instrument at zero. The thiamine content was calculated using the formula:

$$Thiamine\frac{mg}{100} = \frac{100}{W}X\frac{Au}{As}X\frac{C}{1}X\frac{Vf}{Va}X\ D$$

Where: W - Weight of sample analysed, Au = Absorbance of sample, As = Absorbance of standard solution, C = Concentration (mg/mL) of standard solution, Vf= Total volume of filtrate, Va = Volume of filtrate analysed, D = Dilution factor where applicable.

Determination of riboflavin (B_2) content

Exactly 1 gram of sample was weighed into a conical flask and was dissolved with 100 mL of deionized water. This was shaken thoroughly and heated for 5 min and allowed to cool and then filtered. The filtrate was poured into cuvettes and their respective wavelengths for the vitamins set to read the absorbance using spectrophotometer (Wekhe and Chuku, 2021) [58].
Vitamin B2 = 242 nm

$$vitamin\ conc.\left(\frac{mg}{\%}\right) = AxDfxVol.of\ curvette$$

Where: A = Absorbance, E = Extinction co-efficient = 25 for B_2, Df = Dilution factor

Determination of niacin (B_3) content

A measured weight (5 g) of each sample was treated with 50 mL of 1M sulphuric acid (H_2SO_4 solution) and was shaken

for 30 min. The mixture was treated further with 3 drops of aqueous ammonia and filtered. The filtrate (extract) was used for the analysis. Standard niacin (nicotinic acid) solution was prepared and diluted as desired. Exactly 10 mL portion of the standard solution, sample extract and 10 mL of the acid solution (treated with a drop of ammonia) was dispensed into separate flasks to serve as standard, the sample and reagent blank respectively. Each of them was treated with 5 mL of normal potassium cyanide solution and acidified with 5 mL of 0.02 N H_2SO_4 solution; its absorbance was read in a spectrophotometer at a wavelength of 470 nm (Wekhe and Chuku, 2021) [58]. The reagent blank was used to calibrate the instrument at zero. Niacin content was calculated using the formula;

$$Nacine\frac{mg}{100} = \frac{100}{W} X \frac{Au}{As} X \frac{C}{1} X \frac{Vf}{Va} X\, D$$

Where; W - Weight of sample analysed, Au = Absorbance of sample, As= Absorbance of standard solution, C = Concentration (mg/mL) of standard solution, Vf = Total volume of filtrate, Va = Volume of filtrate analysed, D = Dilution factor where applicable, C =Concentration of standard solution.

Determination of ascorbic acid (Vitamin C) content

The method described by AOAC (2010) was used. Exactly 10 g of the sample was extracted with 50 mL EDTA/TCA (50 g in 50 mL of water) extracting Solution for I hour and filtered through a Whatman filter paper into a 50 mL volumetric flask and made up to the mark with the extracting solution. Exactly 20 mL of the extract was pipetted into a 250 mL conical flask and 10 mL of 30 % KI is added and also 50mL of distilled water added. This was followed by 2 mL of 1 % starch indicator. This was titrated against 0.01 mL $CuSO_4$ solution to a dark end point (Sengev *et al*., 2016) [50]

$$Vit.C\frac{mg}{100\ g} = 0.88\, X \frac{100}{5} X \frac{Vf}{20} X \frac{T}{1}$$

Where:
Vf = Volume of extract, T = Sample titre – blank titre.

6. Determination of phytochemical content of the complementary foods

Sample extraction: Sample extraction was carried out based on the method describe by Wahyono *et al*. (2020) [2] with minor modification. Complementary foods were dried in the oven at 40 ºC for 24 hours. Then the complementary foods were mashed. The mashed samples were extracted with 80% methanol with a ratio of 5 grams of formulations and 12.5 mL of methanol. The complementary foods extract was put in a shaker (100 rpm) for 2 hours at 37 ºC. The total phenolic and flavonoid content was determined as follows:

Determination of total phenolic content

Total phenolic content of each extract was determined using the Folin–Ciocalteu method as described by Lilei *et al*. (2013). Briefly, a 10-fold dilution of Folin–Ciocalteu reagent was prepared just prior to use. Then, 1.5 mL of freshly diluted Folin–Ciocalteu reagent was used to oxidize 0.2 mL sample extracts. After allowing the mixture to equilibrate for 5 min, the reaction was then neutralized with 1.5 mL sodium carbonate solution (60 g/L) at room temperature. The absorbance of the resulting solution was measured at 725 nm after 90 min against a blank of acidified ethanol (1 N HCl/95 % ethanol, v/v, 15/85) using a spectrophotometer. Gallic acid was used as a standard. The total phenolic content of complementary foods was expressed as mg of Gallic Acid Equivalent (mgGAE/100 g).

Determination of total flavonoids content

Total flavonoid content was determined by a colorimetric method as described by Chlopicka *et al*. (2012) [14]. Briefly, 0.25 mL of the 80 g/100 g methanolic extract was diluted with 1.25 mL of distilled water. Then 75 mL of 5 g/100 g $NaNO_2$ solution was added to the mixture and after 6 min 150 mL of 10 g/100 g $AlCl_3.6H_2O$ solution was added. The mixture was allowed to stand for 5 min and next 0.5 mL of 1 mol/L NaOH was added and the total was made up to 2.5 mL with distilled water. The solution was mixed well and the absorbance was measured immediately against the blank at 510 nm using a spectrophotometer Jasco UV-530. The results were expressed as mg of Quercetin Equivalent (mgQE/100 g).

7. Antioxidant Activity of the Complementary Foods

Sample extraction: Sample extraction was carried out based on the method of Wahyono *et al*. (2020) [2]. Complementary foods were dried in the oven at 40 ºC for 24 hours. Then the dry complementary foods were mashed. The mashed samples were extracted with 80 % methanol with a ratio of 5 g of formulations and 12.5 mL of methanol. Then samples extract was put in a shaker (100 rpm) for 2 hours at 37 ºC. The antioxidant activity was obtained by determination of DPPH (1,1-DiPhenyl-2-PicrylHydrazil) radical-scavenging capacity.

DPPH Radical Scavenging Activity

The DPPH method was used according to the modified method of Lilei *et al*. (2013). A 60 µmol/L DPPH■ reactant was made in methanol. Then 3.9 mL of DPPH■ solution was added to 0.1 mL of sample, and the absorbance at 515 nm was measured at t = 60 min. To determine the absorbance at t = 0 min, measurement was immediately taken after adding 3.9 mL of DPPH■ solution to 0.1 mL methanol. The antioxidant activity was calculated as:

$$\%\ \text{DPPH}\bullet\ \text{scavenging activity} = \left(1 - \left[\frac{Asample,t}{Acontrol,t} = 0\right]\right) x\ 100$$

A plot of trolox concentration with % DPPH■ scavenging activity was used as the standard curve. Based on this curve, the concentrations of samples were expressed as micromole Trolox Equivalents /g (µmol TE/g).

Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power (FRAP): The reducing property of the extracts was determined by assessing the ability of the extract to reduce ferric chloride ($FeCl_3$) solution as described by Adetuyi & Ibrahim, (2014) [5]. A 2.5 mL aliquot was mixed with 2.5 mL of 200 mm sodium phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min; thereafter 2.5 mL of 10 % trichloroacetic acid was added. This mixture was centrifuged at 2 000 x g for 10

min; 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1 % ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer (JENWAY 6305) and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard and result expressed mg of ascorbic acid (mgAAE/100 g).

Microbial Analysis of the Complementary Foods

Sample processing: Each sample (10.0 g) was homogenized with 90.0 mL of sterile normal saline to prepare stock solution. Stocks were serially diluted 10^1 to 10^5 by adding 100µl of stock solution to 900µl normal saline in eppendorfs tubes. 100µL of diluted sample was inoculated on Nutrient agar (NA), Mannitol Salt Agar (MSA), Violet Red Bile Salt Glucose agar (VRBG) and Sabouraud Dextrose agar (SDA) media following spread plate method and incubated at 37 °C for 18-24 hours except for SDA which was incubated at 25 °C for 48-72 hours. All media were procured from Himedia Laboratories Ltd., India (Khanom *et al*., 2016) [32].

Total Viable Count of the complementary foods

Nutrient agar (NA) media was used to determinate the total bacterial count. NA plates were dried and labeled for appropriate dilutions to be used for dilution and spread plate method. Plates were inoculated and incubated at 37 °C for 18-24 hours. Total number of bacteria cfu/g of sample was calculated and recorded for interpretation of the result by Gernah *et al*. (2011); Khanom *et al*. (2016) [25, 32]; Uchegbu Nneka *et al*. (2019) [40].

Yeast and Molds count

Diluted samples were inoculated onto SDA medium supplemented with cloramphenical (40mg/L) by spread plate method as mentioned earlier. The plates were incubated at 37°C for 48-72 hours. Visible colonies were counted and calculated as the total yeast and mold and recorded as cfu/g (Gernah *et al*., 2011; Khanom *et al*., 2016; Oben *et al*., 2021) [25, 32, 44]

Determination of total Enterobacteriaceae

Violet Red Bile Salt Glucose agar (VRBG) agar was used for propagation of Enterobacteriaceae. After incubation at 37 °C for 24 hours characteristic purple and red colonies were counted as members of Enterobacteriaceae. Suspected colonies were counted to determine cfu per gram of sample (Khanom *et al*., 2016) [32].

Determination of counts of *Staphylococcus aureus*

Mannitol Salt Agar (MSA) was used to determine the counts of *Staphylococcus aureus* in flour samples. The suspected colonies of *Staphylococcus aureus* showed yellow color for mannitol fermentation and yellow halo for coagulase production around the colony. Suspected colonies were further confirmed by catalase, coagulase tests and Gram staining technique (Khanom *et al*., 2016) [32]. Typical S. aureus colonies were counted to calculate cfu per gram of sample

Statistical Analysis

Data obtained was analyzed using one-way ANOVA and mean separated using Duncan Multiple Range Test (DMRT) at 5 % limit of significance using Statistical package for social science (SPSS) version 21 (Eli *et al*., 2022) [22].

Results and Discussion

1. pH and Total Titratable Acidity (TTA) During Fermentation and Malting

Table 2: Change in pH and TTA during fermentation and malting

	Fermentation (cocoyam)		Malting (red kidney beans)	
Time (h)	pH	TTA (%)	pH	TTA (%)
0	6.57^{a}±0.03	0.12^{a}±0.04	6.81^{a}±0.03	0.16^{a}±0.06
24	5.07^{b} ±0.01	1.28^{b}± 0.02	6.78^{a}±0.10	0.25^{a}±0.02
48	4.29^{c} ±0.02	2.92^{c}±0.05	6.72^{a}±0.02	0.91^{a}±0.15
72	4.03^{d}±0.01	3.34^{d}±0.03	6.69^{a}±0.14	0.96^{a}±0.02
96	3.99^{d}±0.01	3.36^{d}±0.02		

Key: TTA: Total Titratable acidity

The variation in pH and Total Titratable acidity (TTA) during fermentation (cocoyam) and malting (red kidney beans) are presented in Table 2. The pH of cocoyam during fermentation ranged from 6.57 (0 h) - 3.99 (96 h). There was decrease in pH with the increased of fermentation time. This could be as a result of the hydrolysis (by lactic acid bacteria and yeasts) of some complex organic molecules such as lipids, protein and phytin to fatty acids, lactic acid, acetic acid, amino acids and phosphate (Tope & Soji, 2013; Sharma *et al*., 2020) [51, 55].

The TTA of the cocoyam during fermentation ranged from 0.12 % (0 h) - 3.36 % (96 h). There was increase in TTA with the increase of fermentation time. The increases in TTA and subsequent decrease in pH had been reported to be associated with fermentation dominated by lactic acid bacteria and yeasts (Saccharomyces cerevisiae) (Tope & Soji, 2013) [55].

The pH of red kidney beans during malting ranged from 6.81 (0 h) - 6.69 (72 h). There was decrease in pH as malting period progressed. During malting, the enzymes debranched amylose and amylopectin chains, increased the amount of reducing sugars essential for fermentation, this explain why malting result to decrease in pH. This is in agreement with study by Mohammed *et al*. (2017) [36], according to their report on Cereal Weaning Foods. The TTA of the red kidney beans during malting ranged from 0.16 % (0 h) - 0.96 % (72 h). There was increase in TTA as malting period progressed due to the hydrolysis of protein and lipids to amino acids and fatty acid respectively. The decreases in pH and the corresponding increases in TTA during fermentation and malting agrees with report presented by Tope & Soji, (2013) [55]; Ocheme *et al*. (2015).

2. Vitamin content of the complementary foods

Table 3: Vitamins content (mg/100 g) of complementary foods

Complementary foods	β-carotene (µg /100 g)	B_1	B_2	B_3	VIT C
FMM	30.04^{b}±0.25	0.37^{b}±0.02	0.41^{a}±0.01	4.17^{a}±0.12	9.85^{a}±0.02
FNMM	22.05^{c}±0.03	0.34^{b}±0.02	0.39^{a}±0.01	4.08^{b}±0.16	7.88^{c}±0.02
NFMM	37.25^{a}±0.03	0.39^{a}±0. 01	0.42^{a}± 0.01	4.14^{c}±0.24	9.02^{b}±0.01
NFNMM	18.55^{d}±0.25	0.32^{c}±0.02	0.37^{b}±0.06	4.06^{c}±0.04	7.43^{d}±0.02
WFP (2018)	300-1250	0.3 - 0.6	0.4- 0.8	4 -10	15-60

Values are means of triplicate determinations ± S.D. Means followed by different superscript letters in the same column indicate significant difference at ($p<0.05$). FMM: Fermented cocoyam/ malted red kidney beans/ mango; FNMM: Fermented cocoyam/ non- malted red kidney beans/ mango; NFMM: Non- fermented cocoyam/ malted red kidney beans/ mango; NFNMM: Non- fermented cocoyam/ Non- malted red kidney beans/ mango, WFP: World food program 2018

Table 3 shows the vitamin content of the complementary foods. The beta –carotene content of fermented and/or malted complementary foods was significantly ($p<0.05$) higher than the Non -malted /Non- fermented (NFNMM) (Table 10). This might be due to malting and fermentation of the raw materials. It is postulated that the observed increase in beta- carotene may be ascribed to its synthesis by microorganism during fermentation (Sengev *et al*., 2016) [50]. The increases in Beta- carotene with increasing malting suggested that higher synthesis and/extractability of β-carotene occurs during malting (Gautam & Gupta, 2018; Onwurafor *et al*., 2020) [24, 47]. Also during malting, the anti-nutrients that chelates or destroys pro-vitamin A, are destroyed (Dioha *et al*., 2023) [18]. Processing methods including microbial fermentation, reduces or neutralizes tannins and phytates, which results in greater bio-availability of vitamins (β-carotene). A similar result of increase in pro-vitamin A content of bambara groundnut and cowpea due to malting has been reported by Attaugwu and Uvere, (2017) [12]. This report agree with report presented by Sengev *et al*. (2016); Gautam & Gupta, (2018); Onwurafor *et al*. (2020) [24, 47, 50] but the values are relatively higher due to the further addition of mango flour. Beta carotene content of fermented and/or malted complementary foods were not within the world food program recommendations for weaning foods (WFP, 2018)

The vitamin B_1 content of fermented and/or malted samples was significantly (<0.05) higher than that of non-fermentation / non- malting (NFNMM) complementary foods (Table 3). This might be due to fermentation and malting. The increase in vitamin B_1 could be attributed to activities of microorganisms during fermentation. Processing methods including microbial fermentation, reduces or neutralizes tannins and phytates, which results in greater bio-availability of vitamins B complex. Murdock and Fields (1984) [39] reported that the levels of vitamin B_1 and folic acid were increased by lactic acid fermentation of maize. Mostafa *et al*. (2013) [37] also reported the production of vitamin B_1 by Klebsiella pneumonia during solid state fermentation. This might also be due to biosynthesis by germinating seeds (new sprouts) (Kim *et al*., 2012; Ogbonna *et al*. 2012; Onyango *et al*. 2013; Wang *et al*. 2014; Zilic *et al*., 2015; Oghbaei & Prakash, 2016 [28, 33, 45, 48, 62]; Ojha *et al*. 2018). This report agrees with findings presented by Oghbaei & Prakash, (2016) [45],who noted that malting increased the vitamin content of food products. Also (Tope & Soji, 2013) [55], reported that during fermentation, lactic acid bacteria and yeasts in which the former was responsible for an acid environment which was favorable for the proliferation of yeasts while the latter produced vitamin. The addition of mango flour further improved the vitamin B_1 content of the complementary foods. Vitamin B_1 content of the complementary foods was within world food program (WFP, 2018) recommendation from complementary foods (WFP, 2018).

Riboflavin content of the fermented and/or malted complementary foods was significantly higher than that of non- fermented / non malted (NFNMM). This might be due to fermentation and malting (Oghbaei & Prakash, 2016). According to, Tope and Soji, (2013); Sharma *et al*. (2020) [45, 51, 55], during fermentation lactic acid bacteria and yeasts in which the former was responsible for an acid environment which was favorable for the proliferation of yeasts while the latter produced vitamin. This might also be due to biosynthesis by germinating seeds (new sprouts) (Zilic *et al*., 2015; Kaur *et al*., 2020) [31, 62]. This report agrees with findings presented by Oghbaei & Prakash, (2016); Gautam & Gupta, (2018) [24, 45], who noted that malting increased vitamin B_2 content of food products. Vitamin B_2 content of the complementary foods was within World Food Program recommendation from complementary foods (WFP, 2018).

Vitamin B_3 content of the fermented and/or malted complementary foods was significantly ($p<0.05$) higher than that of non- fermented /non malted complementary food (Table 3). This might be due to fermentation and malting (Oghbaei & Prakash, 2016) [45]. This might also be due to biosynthesis by germinating seeds (new sprouts) (Zilic *et al*., 2015; Kaur *et al*., 2020). (Tope & Soji, (2013) [31, 55, 62] also reported that during fermentation, lactic acid bacteria and yeasts in which the former was responsible for an acid environment which was favorable for the proliferation of yeasts while the latter produced vitamin. This report agrees with findings presented by (Oghbaei & Prakash, 2016), Gautam & Gupta, (2018) [24, 45], who noted that malting increased vitamin B_3 content of food products. Vitamin B_3 content of the complementary foods was within World Food Programe recommendation from complementary foods (WFP, 2018). B complex help prevent infections and help support or promote: growth of red blood cells, proper nerve function and brain functioning.

Vitamin C content of the fermented and/or malted weaning foods was significantly ($p<0, 05$) higher than the Non fermented / non- malted complementary foods (Table 10). This might be due to malting and fermentation of the raw materials (Kiptanui *et al*., 2022) [9]. Fermentation has been noted to improve the ascorbic acid content of foods may be due to the activity of microorganisms (lactic acid bacteria's and yeast) (Desai *et al*. 2010, Adetuyi & Ibrahim, 2014 [5];

Laxmi *et al*. 2015; Gautam & Gupta, 2018) [24]. This report agrees with results presented by Adetuyi & Ibrahim, (2014); Gautam & Gupta, (2018) [5, 24]. The increase in vitamin C during malting/germination is driven by enzymatic hydrolysis of starch by amylases and diastases that increase availability of glucose for the biosynthesis of vitamin C. It is this enhanced content of glucose that acts as a precursor to the formation of vitamin C (Desai *et al*., 2010). This postulation is supported by work done by (Nkhata *et al*., 2018) [42], who carried out a study on rats. They reported that D-glucose is converted into D-glucuronolactone which then changes to L-gluconolactone and finally into L-ascorbic acid. This study confirmed that C-6 of glucose could be oxidized to form the carboxyl carbon of the ascorbic acid. Nkhata *et al*. (2018) [42] concluded that the same could happen in plants during fermentation or malting. The addition of mango flour further improved the vitamin C content of the complementary foods. The vitamin C content of the complementary foods was not within the world food program recommendations.

Vitamin C is the most well-known antioxidant. With its antioxidant properties, this vitamin provides a protective role in cardiovascular disease. Zhang *et al*. (2015), in a study conducted on cigarette smokers, demonstrated that ascorbic acid in conjunction with other antioxidants (including vitamin E) inhibited elevated markers of lipid peroxidation induced by smoking in smokers. Antioxidants, including vitamin C, were confirmed to have a protective role in coronary heart disease and cardiovascular disease. Based on its antioxidant capacity, ascorbic acid protects body cells from oxidative stress (Gautam & Gupta, 2018) [24].

3. Phytochemical content and antioxidant capacity of complementary foods

Table 4: Phytochemical content and antioxidant capacity of complementary foods

Complementary foods	Total phenolic (mgGAE/100 g)	Total Flavonoids (mgQE/100 g)	DPPH (µmol TE/100 g)	FRAP (mgAAE/100 g)
FMM	4.23^{a}±0. 03	8.31^{a}± 0.01	31.46^{d}±0.04	14.26^{a}±0.02
FNMM	2.64^{c}±0.01	6.02^{c}±0.54	28.36^{b}±0.34	8.87^{c}±0.01
NFMM	3.86^{b}±0.04	6.26^{b}±0.02	26.68^{c}±0.67	9.13^{b}±0.05
NFNMM	2.16^{d}±0.43	5.31^{d}±0.52	41.74^{a}±0.13	6.75^{d}±0.04

Values are means of triplicate determinations ± S.D. Means followed by different superscript letters in the same column indicate significant difference at ($p<0.05$). GAE – Gallic Acid Equivalen, QE – Quercetin Equivalent, TE = Trolox Equivalents, AAE - Ascorbic Acid Equivalent.

FMM: Fermented cocoyam/ malted red kidney beans/ mango; FNMM: Fermented cocoyam/ non- malted red kidney beans/ mango; NFMM: Non- fermented cocoyam/ malted red kidney beans/ mango; NFNMM: Non- fermented cocoyam/ Non- malted red kidney beans/ mango, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, FRAP: Ferric reducing antioxidant power

As shown in Table 4, phenolic content of the treated and/ or malted complementary foods (FMM, FNMM, NFMM) was significantly ($p<0.05$) higher than the non-fermented/ non malted complementary food (NFNMM). Increase phenolic could be due to synthesis or transformation of other compounds during germination (Tarasevičienė *et al*., 2019; Kaur *et al*., 2020; Winarsi *et al*., 2020) [31, 53]. Transformation of tannins to phenols (phenyl oxidase action) occurring during fermentation (Nkhata *et al*., 2018) [42]. PAL, the enzyme that catalyzes the pathways responsible for biosynthesis of the different phytochemicals (phenolic acids and flavonoids) is activated during germination (Nkhata *et al*., 2018) [42]. Another possible explanation is that there could be hydrolysis of bound phenolic compounds during fermentation and malting; that is phytase breakdown complex matric freeing phytochemicals (phenols) (Onyango *et al*., 2013; Zilic *et al*., 2015; Nkhata *et al*., 2018; Sharma *et al*., 2020) (Cruz-Casas *et al*., 2021) [20, 42, 48, 51, 62].This result is in agreement with reports by Gautam & Gupta, (2018); Nkhata *et al*. (2018);Verni *et al*. (2019); Adebo & Medina-Meza, (2020) [3, 24, 57].

As presented in Table 4, flavonoids content of fermented and/or malted complementary foods (FMM, FNMM, NFMM) was significantly ($p<0.05$) higher than that of non-fermented/ non- malted complementary food (NFNMM). It indicated that fermentation and malting could enhance the release of bound flavonoid components; that is phytase breakdown complex matric freeing phytochemicals (flavonoids) (Zhang *et al*., 2015; Sharma *et al*., 2020; Winarsi *et al*., 2020; Alrosan *et al*., 2022) [9, 51, 59]. This is in agreement with reports by Guzmán-Uriarte *et al*., (2013); Adetuyi & Ibrahim, (2014); Gautam & Gupta, (2018); Nkhata *et al*. (2018); Verni *et al*. (2019) [5, 24, 26, 57]; Samtiya *et al*. (2020). Another possible explanation is that there could be novo biosynthesis of phenols in the embryonic axis of the sprouts (Onyango *et al*., 2013; Zilic *et al*., 2015) [48, 62].

In the presence of hydrogen donors, DPPH (2, 2-diphenyl-1-picrylhydrazyl) is reduced and a free radical is formed from the scavenger. The reaction of DPPH is monitored by the decrease of the absorbance of its radical at 517 nm, but upon reduction by an antioxidant, the absorption disappears. The lower value indicates stronger antioxidant activity in the sample. As presented in Table 4, the radical scavenging ability of fermented and/or malted complementary foods (fermented and/or malted) was significantly ($p < 0.05$) higher than that of non- fermented/ non- malted complementary food (NFNM). Several researchers have found out that fermentation and malting does increase the DPPH radical-scavenging ability (Guzmán-Uriarte *et al*., 2013; Adetuyi & Ibrahim, 2014; Gautam & Gupta, 2018; Nkhata *et al*., 2018) [5, 24, 26]. This could be due to the release of bounded phenolics, Flavonoids, bioactive peptides, vitamin C & E by the catalytic action of β glucosidase during fermentation and the formation reductones during fermentation could contribute to such increase in antioxidant activities. Since red kidney beans and cocoyam have been found to contain protein, the breakdown of protein to free amino acids and peptides (bio-active peptides) by microbial protease activity and hydrolytic enzymes (proteolysis) could

also account for the increase in the antioxidant activity (Riciputi *et al*., 2016; Verni *et al*., 2019) [49, 57]. Also lactic acid bacteria (LAB) present in fermented products have been to exhibit antioxidant capacity (Feng & Wang, 2020) [23]

Antioxidants can be used to delay the oxidative rancidity of fats in stored foods, thereby maintaining product appeal and extending shelf-life. The high antioxidant capacity fermented and/or malted complementary foods was implies delay oxidative rancidity and extended shelf- life compared to non- fermented / non – malted (Tonolo *et al*., 2019) [54]. Also the high antioxidant capacity of fermented and/ or malted complementary foods implies high capacity to reduce oxidative stress and free radicals in the body compared to non- fermented / non- malted complementary foods (Feng & Wang, 2020) [23].

The reducing powers of the complementary foods were assessed based on their ability to reduce Fe^{3+} to Fe^{2+} is presented in Table 4. The reducing power of the fermented and/or malted complementary foods was significantly ($p < 0.05$) higher than that of non-fermented/ non- malted complementary food (NFNM). It shows that fermentation and malting enhance the release of several classes of phenolics phytochemicals, ascorbic acid and α- tocopherol (vitamin E) (Moongngarm & Saetung, 2010). Flavonoids, polyphenolic and ascorbic acid are compounds known for their high antioxidant properties and free radical scavenging ability (Xu *et al*., 2021). Since cocoyam and red kidney beans have been found to contain protein, the breakdown of protein to free amino acids and peptides (bio-active peptides) by microbial protease activity could also account for the increase in the antioxidant activity (Riciputi *et al*., 2016; Verni *et al*., 2019) [49, 57]. This report is in agreement with reports by Guzmán-Uriarte *et al*., (2013); Adetuyi & Ibrahim, (2014); Gautam & Gupta, (2018); Nkhata *et al*. (2018); Verni *et al*. (2019) [5, 24, 26, 57].

4. Microbiological quality of the complementary foods

Table 5: Microbiological quality (**cfu/g)** of the complementary foods

Complementary foods	Total viable count (TVC)	YMC	EC	Tsac
FMM	$2.20^{a}x10^{1}$	$1.70^{a}x10^{1}$	Nill	Nill
FNMM	$2.30^{a}x10^{1}$	$1.40^{b}x10^{1}$	Nill	Nill
NFMM	$1.20^{b}x10^{1}$	$1.30^{c}x10^{1}$	Nill	Nill
NFNMM	$1.10^{b}x10^{1}$	$1.10^{d}x10^{1}$	Nill	Nill
ICMS limit	0-103	$<10^{2}$	$<10^{2}$	$<10^{2}$

Values are means of triplicate determinations ± S.D. Means followed by different superscript letters in the same column indicate significant difference at ($p<0.05$). FMM: Fermented cocoyam/ malted red kidney beans/ mango; FNMM: Fermented cocoyam/ non- malted red kidney beans/ mango; NFMM: Non- fermented cocoyam/ malted red kidney beans/ mango; NFNMM: Non- fermented cocoyam/ Non- malted red kidney beans/ mango; Cfu/g: colony forming unit per gramme, YMC: Yeast and mould Count, EC: Enterobacteriaecae Count, Tsac: Total *Staphylococcus aureus* Count, Nill: Not Identified; ICMS: International Commission for microbiological specification for foods

The total aerobic or viable count of the complementary foods is shown in Table 5. Fermentation resulted in a significant ($p < 0.05$) increase in total aerobic counts. This increase might be due to the acidic medium created by the pres

References

There are no sources in the current document.

ence lactic acid bacterial (*lactobacillus plantarum, lactobacillus fermentum*) which encourage the growth microorganisms (Ariahu *et al*., 1999; Tope & Soji, 2013; Kure & Wyasu, 2013; Adetola *et al*., 2018; Eli *et al*., 2022) [4, 11, 22, 35, 55]. Fermentation prevents the growth of pathogenic bacteria, making fermented foods safe. This is due to the generation of antimicrobial substances, like bacteriocin, and lower pH, which hinders the survival of pathogens (Nyanzi & Jooste, 2012) [43]. Report according to Tope & Soji, (2013); Nneka *et al*. (2019) [40, 55] described the lactic acid bacteria as the dominant organisms in cereal, legumes, roots and tubers fermentation. The total viable count from this study was similar to those reported by Ariahu *et al*. (1999) and Nneka *et al*. (2019) [11, 40], was within ICMS limit (Nigusse *et al*., 2019) [41] and some will still be destroyed during cooking.

Yeast and mould counts of the complementary foods are shown in Table 5. Fermentation and malting resulted in a significant ($p < 0.05$) increase in yeast and mould counts ranging from $1.04x10^{1}$CFU/g in NFNMM - $1.50x10^{1}$ CFU/g in FMM. This increase may be due to the decreased in pH which made the environment conducive for yeast growth (Adetola *et al*., 2018) [4]. Symbiotic relationships exist between yeasts and lactic acid bacteria during fermentation, the bacteria provide the rapid acidic environment and the yeast provide essential metabolites such as pyruvates, vitamins and amino acids for the bacteria (Adetola *et al*., 2018) [4]. However, the high temperature of cooking is expected to reduce the microorganisms present.

Enterobacteria, Staphylococcus aureus was not detected in the complementary foods. *Staphylococcus aureus* and *Enterobacteria,* are foodborne Pathogens. Food contamination with Enterobacteria and Staphylococcus aureus originate from fecal matter. Good hygiene practices on the part of personnel involved in food preparation eliminate these microbes (Agbemafle *et al*., 2020) [7]

Conclusion

Good vitamin, phytochemical, and antioxidant quality products, which could be used as a complementary food, can be formulated from cocoyam, red kidney beans and mango using germination and/or fermentation unit operations. Fermentation and germination increased the vitamin, phytochemical and antioxidant quality of the product. The microbial properties of porridges from flours blends were within International Commission for microbiological specification for foods.

Disclaimer (artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Competing interests
Authors have declared that no competing interests exist.

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