Effect of Partial Replacement of Wheat Flour with High Quality Cassava Flour on the Chemical Composition, Antioxidant Activity, Sensory Quality, and Microbial Quality of Bread

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ABSTRACT: In the current study, wheat flour was mixed with high quality cassava flour (HQCF) in several ratios: 90:10, 80:20, 70:30, and 60:40, and used to prepare 10%, 20%, 30%, and 40% National Root Crops Research Institute (NRCRI) cassava bread, respectively. 100% wheat bread was prepared as a control (100% wheat bread). Five bread samples were prepared per group. Antioxidant assays [i.e., 2,2-diphenyl- 1-picrylhydrazyl radical (DPPH) scavenging assay, reducing power assay] revealed that the bread samples had considerable antioxidant capacities. Substitution of wheat flour with HQCF at various concentrations resulted in dose dependent decreases in the mineral and protein contents of the resulting bread samples. The crude fiber content of the bread samples was minimal, while the carbohydrate content of the bread samples ranged from 43.86% to 48.64%. A 20% substitution of wheat flour with HQCF yielded bread samples with a general acceptability that was comparable to that of 100% wheat bread. The mean bacteria counts of the bread samples ranged from 2.0×10^3 CFU/mL to 1.4×10^4 CFU/mL, while the fungal counts ranged from 0 CFU/mL to 3×10^3 CFU/mL. There was a positive correlation between the DPPH antioxidant activities and the reducing powers of the bread samples ($R^2 = 0.871$) and a positive correlation between the DPPH antioxidant activities and the flavonoid contents of the bread samples ($R^2 = 0.487$). The higher microbial load of the NRCRI cassava bread samples indicates that these bread samples may have a shorter shelf life than the 100% wheat bread. The significant positive correlation between total flavonoid content and reducing power ($R^2 = 0.750$) suggests that the flavonoids present in the lipophilic fractions of the bread samples could be responsible for the reductive capacities of the bread samples.

Keywords: high quality cassava flour, bread sample, wheat flour, microbial load

INTRODUCTION

Bread is an important staple food in Nigeria, and its consumption is increasing. However, bread is relatively expensive because it is made from imported wheat that is not grown extensively in Nigeria for climatic reasons. As a result of the increased rate of bread consumption in Nigeria, the country has to depend on American red winter wheat for the production of bread and other confectioneries. Nigeria imports over one million tons of American red winter wheat annually, making Nigeria one of the largest importers of American red winter wheat in the world (1-3). This situation has placed a huge burden on the Nigerian economy. Another challenge that has been associated with the continuous use of wheat in bread making is the development of celiac disease (4).

Thus, an alternative source of flour that can be used to produce bread that is similar to or better than bread produced with wheat flour will be a welcomed development.

Cassava (*Manihot esculenta* Crantz) is one of the most important crops in Africa, and Nigeria is the leading producer globally. Cassava tubers can be kept in the ground for up to two years prior to harvesting, but once harvested, they begin to deteriorate because of the high moisture content of the fresh roots (5). To prevent early deterioration, and because of its bulky nature, cassava is usually traded in a processed form, (5) such as high quality cassava flour (HQCF), which is a major intermediate product.

In 2003, a presidential initiative was launched in Nigeria with the aim of adding HQCF (10% w/w) to the

wheat flour used in bread. The purpose of this initiative was to restrict the outflow of funds for the importation of wheat (6) and to encourage research on cassava/wheat composite breads.

In previously published works, different wheat flours were diluted with various proportions of cassava starch and flour (7). Defloor et al. (8) and Khalil et al. (9) reported that the 30% (w/w) inclusion of cassava flour into wheat flour could yield an acceptable fresh loaf of bread, depending on the source of the flour. Recently, International Institute of Tropical Agriculture (IITA) reported that they successfully produced 40% cassava flour bread that had similar eating qualities to 100% wheat flour bread. This innovation, if adopted, will help Nigeria-Africa's largest oil producer-save about $252 million annually and improve the livelihoods of cassava farmers in the country (10).

Despite efforts by the Nigerian government to sustain HQCF technology, which has a significant economic advantage, many Nigerians are hesitant to incorporate different levels of HQCF into wheat flour for bread making.

Those in the bakery sector have given several reasons for the slow adoption of this technology, including ease of deterioration of cassava bread due to its high moisture and microbial load (personal communication), fear of the presence of toxic components in cassava, and bulkiness.

According to World Health Organization (11), foodborne diseases undermine not only human health and productivity, but also countries' potential for sustainable development. Previous investigations into the incorporation of HQCF into wheat flour for bread making have concentrated on the acceptability of the composite bread to consumers. To answer the questions raised by bakers, we investigated the effects of the substitution of wheat with cassava on microbial load, chemical composition, antioxidant activities, and consumer acceptability of cassava/wheat composite breads.

MATERIALS AND METHODS

The cassava samples used for this study were harvested from the experimental farm of the National Root Crops Research Institute (NRCRI) in Umudike, Nigeria. Har-

vested samples were peeled, thoroughly washed (manually), and grated into a mash with a grater. The mash was dewatered by manual pressing in clean jute bags, the lumps were disintegrated, and the mash was sundried on a raised platform for about 5 h. A Cassava Hammer Mill (Large Handling Capacity Cassava Grinding Hammer Mill, JH, Henan, China) was used to mill the dried mash into flour with a particle size of about 2 mm, and the resulting flour was sieved with muslin cloth to obtain HQCF. The resulting HQCF was incorporated into wheat flour at different levels of substitution and used to make bread.

Preparation of composite flours
Wheat flour was mixed with HQCF at several ratios: 90:10, 80:20, 70:30, and 60:40 at the Post Harvest Technology Program of the NRCRI. The 90:10, 80:20, 70:30, and 60:40 flour mixtures were used to prepare 10%, 20%, 30%, and 40% NRCRI cassava bread, respectively. The control bread was prepared with 100% wheat flour. Five samples of bread were prepared per group.

Preparation of dough
The 100% wheat flour dough was prepared according to the method described by Aniedu and Oti (12), as shown in Table 1.

pH
A 10% (w/v) suspension of bread in distilled water was prepared for each bread sample. The suspension was mixed thoroughly in a food blender (One-Gallon 3.75 HP Variable-Speed Food blender CB15V, Waring Commercial, Torrington, WY, USA) and the pH was measured with a pH meter (HI98127, Hanna Instruments, Inc., Carrollton, TX, USA).

Proximate analysis
The moisture, ash, lipid, and protein contents of the bread samples were determined using the method of the Association of Analytical Chemists (13). The carbohydrate content of the samples was calculated by difference. The Atwater system was used to calculate the energy value of each sample. Briefly, carbohydrate energy values were calculated by multiplying the total carbohydrate content of each sample by 4, lipid energy values

Table 1. Composition of dough (unit: g)

Samples	CF	WF	Sugar	Margarine	Yeast	Salt	Nutmeg	Water
Control	0	1,200	100	120	3	Pinch	0.25	As needed
10%	120	1,080	100	120	3	Pinch	0.25	As needed
20%	240	960	100	120	3	Pinch	0.25	As needed
30%	360	840	100	120	3	Pinch	0.25	As needed
40%	480	720	100	120	3	Pinch	0.25	As needed

CF, cassava flour; WF, wheat flour.

were calculated by multiplying the lipid content of each sample by 9, and protein energy values were calculated by multiplying the protein content of each sample by 4. Energy values were expressed as kcal/100 g.

Determination of alkaloid content

The gravimetric method of Harborne (14) was used to determine the total alkaloid content of the bread samples. Briefly, 5 g of each sample was added to 50 mL of 10% acetic acid in ethanol. The mixture was shaken well and allowed to stand for 4 h before filtering. The filtered sample was evaporated to one quarter of its original volume and drops of ammonium hydroxide were added to precipitate the alkaloids. The precipitate was filtered off with a pre-weighed filter paper and washed with a 1% ammonium hydroxide solution. The precipitate was oven dried for 30 min at 60°C and then weighed. The alkaloid content of the samples was determined by the following equation:

$$\text{Percent alkaloid} = \frac{W_2 - W_1}{W} \times 100$$

where W is initial weight of sample, W_1 is weight of empty filter paper, and W_2 is weight of paper+precipitate.

Determination of flavonoid content

Ten grams of each sample were extracted repeatedly with 100 mL of 80% methanol at room temperature. The mixture was filtered through Whatman No. 1 filter paper. The filtrate was transferred to a crucible, evaporated to dryness over a water bath, and weighed (15).

Determination of cyanide content

The alkaline picrate method (16) was used to determine the cyanide content of each bread sample. A portion (5 g) of each sample was ground into a paste, dissolved in 50 mL of distilled water in a corked conical flask, allowed to incubate for 12 h, and filtered. The filtrate was used for cyanide determination. Briefly, 4 mL of alkaline picrate, which was prepared by dissolving 1 g of picric acid and 5 g of Na_2CO_3 in 100 mL of distilled water, was added to 1 mL of the sample filtrate in a corked test tube. The mixture was incubated in a water bath for 5 min until a reddish-brown color developed. After stabilizing the spectrophotometer with a blank (i.e., 1 mL of distilled water and 4 mL of alkaline picrate), the absorbance of the reddish-brown solution was read at 490 nm. The cyanide content of the solution was extrapolated from a cyanide standard curve, and the following formula was used to calculate the cyanide concentration of each bread sample:

$$\text{Cyanide (mg/kg)} = \frac{\text{Absorbance} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of the sample}}$$

Determination of tannins

The tannin concentration of each bread sample was determined using the method of AOAC (13). Tannin contents were calculated from a tannic acid standard curve, and results were expressed as milligrams of tannic acid equivalents (TAE) per 100 g of dried sample.

Phytate determination

Phytate was extracted with trichloroacetic acid and precipitated as ferric salt using the method of Wheeler and Ferrel (17).

Oxalate determination

The oxalate content of each bread sample was analyzed using the calcium oxalate precipitation method (13).

2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging assay

A modified version of the method of Blois (18) was used to determine DPPH radical scavenging ability. Briefly, 5 g of each sample was dissolved in 100 mL of methanol to give a concentration of 50 mg/mL. The mixture was filtered through Whatman No. 1 filter paper with a vacuum pump. Then, 10 μL, 20 μL, 30 μL, 40 μL, and 50 μL aliquots of each extract were further diluted with methanol to give final concentrations of 50 μg/mL, 100 μg/mL, 150 μg/mL, 200 μg/mL, and 250 μg/mL, respectively. Finally, 0.1 mL of 0.3 mM DPPH (in methanol) was added to each of the reaction mixtures and the whole setup was shaken well and left in the dark for 30 minutes. After the incubation period, the spectrophotometer was stabilized against a DPPH control (i.e., 1 mL of methanol), and the absorbance of each sample mixture was read spectrophotometrically at 517 nm. The scavenging activity was calculated with the following equation:

$$\text{Percent scavenging activity} = \{(\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control}\} \times 100.$$

Reducing power assay

A modified version of the method of Hsu et al. (19) was used to determine the reducing power of each bread sample. Briefly, 2 g of each bread sample was dissolved in 40 mL of methanol to give a stock concentration of 50 mg/mL. The mixture was left overnight and then filtered through Whatman No. 1 filter paper with a vacuum pump. The filtrate was brought up to 50 mL with methanol to give a concentration of 40 mg/mL and then diluted to the following concentrations: 5 mg/mL, 10 mg/mL, 15 mg/mL, 20 mg/mL, and 25 mg/mL. The resulting solutions were used for the reducing power assay. Briefly, 1 mL of extract, 0.5 mL of phosphate buffer (0.2 M, pH 6.6), and 2.5 mL of potassium ferricyanide (instead of potassium hexacyanoferrate) solution (1% v/v in water)

were placed in a test tube and incubated for 20 min at 50°C. The tubes were cooled in crushed ice and 0.5 mL of trichloroacetic acid (10% in water) was added to each tube. After centrifugation at 3,000 g for 10 min, 1 mL of the supernatant was mixed with an equal volume of distilled water and 0.1 mL of ferric chloride solution (0.1% in water). The mixture was incubated for 10 min and the absorbance was measured at 700 nm using a UV spectrophotometer. A higher reaction mixture absorbance indicates a greater reducing power.

Microbial assay
The total viable count of each bread sample was determined using the pour plate technique (20). Briefly, 10 g of each sample was homogenized in 90 mL of sterile peptone water in a sterile 500 mL gas jar cylinder. The solution was agitated vigorously for a few minutes to ensure even mixing and then allowed to settle. A ten-fold dilution series was performed to obtain a colony count of 1×10^{-3}/mL. One mL of the diluted sample was poured into an empty, sterile 9 mL petri dish. Potato dextrose agar (PDA) was poured into the prepared plates for fungi counts, nutrient agar (NA) was poured into the prepared plates for bacterial counts, and MacConkey agar (MA) was poured into the prepared plates for enteric bacteria counts. The PDA media was modified with streptomycin to inhibit bacterial contamination. All of the plates were allowed to set and then the NA and MA plates were transferred to a 37°C incubator at for 24~48 h, while the PDA plates were transferred to another incubator for 3~5 days. Colonies were counted and recorded after each incubation period. The different colonies on each plate were isolated, purified, and stored on NA slants (for bacteria) and PDA slants (for fungi) for further characterization and identification. The bacteria were identified with a series of cultural and biochemical tests as described by Buchannan and Gibbons (21), while the fungi were identified by cultural and morphological characteristics based on mycelium structure, branch conditions, conidiophore presence, shape, etc. as described by Barnet and Hunter (22).

Sensory evaluation
A trained panel of 15 people evaluated the sensory attributes of the bread samples. Appearance, flavor, taste, texture, and general acceptability were assessed. The scores were based on a seven point hedonic scale, where 7 represented excellent and 1 represented very poor.

Statistical analysis
The statistical package for social sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA) was used to analyze all data.
    Results are presented as mean±standard deviation.

One-way analysis of variance (ANOVA) and Duncan's multiple range test were used for comparison of means. Differences between means were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

The compositions of the bread samples tested in this study (Table 1) were comparable with other breads produced from other countries.
    A pH of 4 or less indicates an appreciable level of fermentation and hence starch breakdown (23). Therefore, the higher the fermentation, the lower the pH of food substances. According to Ihediohanma (24), the glycemic index of foods increases with increased fermentation. In addition, wheat flour is reported to have a higher glycemic index than cassava flour (25). As shown in Fig. 1, all of the NRCRI cassava bread samples had higher pH values than the 100% wheat bread.
    The DPPH assay is a widely accepted method for the determination of the antioxidant activities of various foods. This method is widely used because of the relatively short time required for the analysis. However, the DPPH assay is limited to neutral and higher pH applications and is affected by sample color (26). In this study, the total antioxidant activity of the bread samples decreased in the following order: quercetin standard >100% wheat bread >10% NRCRI cassava bread >40% NRCRI cassava bread >20% NRCRI cassava bread >30% NRCRI cassava bread (Fig. 2). The total antioxidant activity of the samples tested decreased with increasing substitution of wheat flour with HQCF, except at the 40% substitution level used in the 40% NRCRI cassava bread.
    Reducing power assay is another widely accepted method that is used in the determination of the antioxidant activities of various plants. This principle of this assay involves the reduction of Fe^{3+} to Fe^{2+} through
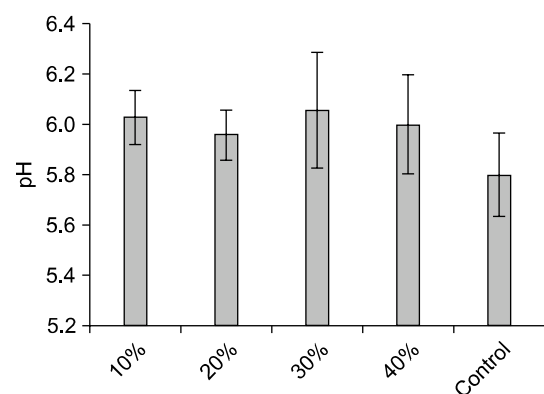

Fig. 1. pH of bread samples. 10%~40%, bread containing 10%~40% NRCRI cassava flour; Control, 100% wheat flour.

electron transfer (27). As shown in Fig. 3, the reducing power of the samples tested decreased in following the order: quercetin standard> 100% wheat bread> 10% NRCRI cassava bread> 40% NRCRI cassava bread> 20% NRCRI cassava bread> 30% NRCRI cassava bread. Similar to the results of the DPPH antioxidant activity assay, the reducing power of the samples tested in this study decreased with increasing substitution of wheat flour with HQCF, except at the 40% substitution level used in the 40% NRCRI cassava bread. The results of this study indicate that wheat flour is a better source of antioxidants than cassava flour.

The ash content of a sample is a measure of its total inorganic mineral content. As shown in Table 2, the incorporation of 10%, 20%, 30%, and 40% HQCF into the wheat flour used for bread making resulted in dose-dependent decreases in the mineral contents of the bread samples tested.

The moisture values of all of the bread samples were high. The baking temperature (175°C to 200°C) and baking time ($\geq$ 20 min) used in this study led to a loss of appreciable amounts of moisture compared to the raw samples. However, different food substances have different capacities for absorbing/retaining moisture, leading to occluded or absorbed water. Therefore, it can be deduced that, even at the high baking temperature used in this study, some moisture will still be found in some samples (28).

The incorporation of 10%, 20%, and 40% HQCF into wheat flour for bread making decreased the lipid content of the dough. However, 30% cassava substitution was associated with increased lipid content (Table 2). All of the NRCRI cassava bread samples had lower lipid contents than the 100% wheat bread.

Crude fiber, which consists of cellulose and lignin, is used as an indicator of dietary fiber content. Dietary fiber is the indigestible/unavailable carbohydrate present in the diet. Diets rich in dietary fiber decrease the reabsorption of bile acids, thus reducing circulating cholesterol levels and increasing glucose tolerance (29). Crude fiber was not detected in the 10%, 30%, or 40% NRCRI cassava bread samples or in the 100% wheat bread. However, the crude fiber content of the 20% NRCRI cassava bread was detectable, but quite low (Table 2).

Substitution of wheat flour with varying levels of HQCF (i.e., 10%, 20%, 30%, and 40%) for the production of NRCRI cassava bread samples resulted in a dose dependent decrease in the protein content of the bread samples (Table 2). However, the 100% wheat bread con-
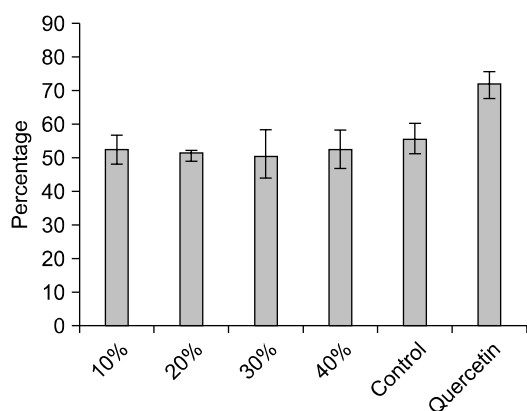

Fig. 2. Scavenging activities of bread samples and quercetin on DPPH radical. 10%~40%, bread containing 10%~40% NRCRI cassava flour; Control, 100% wheat flour.
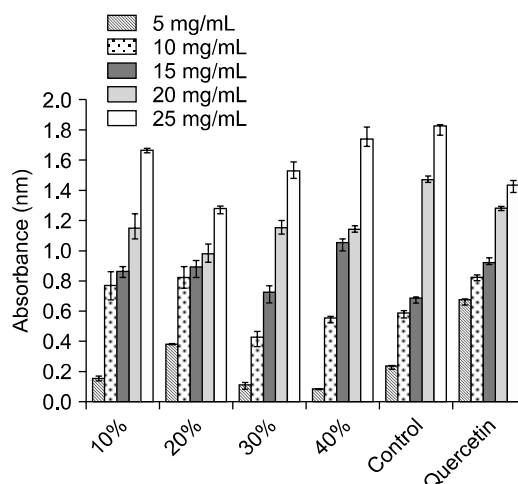

Fig. 3. Reducing power of bread samples and quercetin. 10%~40%, bread containing 10%~40% NRCRI cassava flour; Control, 100% wheat flour.

Table 2. Proximate composition of bread samples

Sample	Ash (%)	MC (%)	Fat	CF (%)	Protein (%)	CHO (%)	EV (kcal/100 g)
Control	1.65±0.09[b]	35.37±1.75[a]	2.67±0.06[e]	<LOD	11.18±0.80[a]	44.38±0.52[a]	265.27±0.72[c]
10%	1.44±0.77[b]	37.23±1.49[ab]	1.97±0.17[c]	<LOD	14.26±0.30[b]	45.44±1.21[ab]	256.53±8.61[bc]
20%	1.40±0.14[b]	38.53±0.62[b]	1.57±0.12[b]	0.07±0.03	13.45±0.58[b]	45.05±1.04[ab]	248.13±6.38[ab]
30%	1.35±0.11[b]	39.79±2.72[b]	2.40±0.21[d]	<LOD	11.68±0.67[a]	44.78±2.37[a]	247.44±5.86[ab]
40%	0.75±0.24[a]	39.72±1.87[b]	1.03±0.08[a]	<LOD	11.18±0.80[a]	47.87±0.77[b]	243.27±5.52[a]

Values are the mean±SD of three determinations (n=5).
[a-e]Within each column, means with different superscripts are significantly different ($P<0.05$).
Control, 100% wheat bread; 10%~40%, bread containing 10%~40% NRCRI cassava flour.
MC, moisture content; CF, crude fiber; CHO, carbohydrate; EV, energy value; LOD, level of detection.

tained higher amounts of protein than the NRCRI cassava bread samples investigated. This is attributed to the higher protein content of wheat flour compared to cassava flour.

The carbohydrate content of the bread samples ranged from 42.91% to 47.69% (Table 2). Higher carbohydrate values were present in the NRCRI cassava bread samples than in the 100% wheat bread. This is attributed to the higher carbohydrate content of cassava flour compared to wheat flour.

The energy value of the bread samples decreased with increasing substitution with HQCF (Table 2). This could be attributed to the decrease in the concentration of lipids, which contribute significantly to the energy value of food samples that was observed with increasing HQCF substitution. All of the NRCRI cassava bread samples had lower energy values than the 100% wheat bread, which indicates that cassava/wheat composite bread may be better for diabetics than 100% wheat bread.

Polyphenols are natural phytochemical compounds that are present in plants and affect the taste, odor, color, nutrition, and antioxidant properties of plant-based foods (30). These polyphenolic compounds also have biological properties, including anti-diabetic, anti-mutagenic, anti-inflammatory, antimicrobial, and anti-carcinogenic effects. The polyphenolic compounds that have been found in plants include flavonoids, alkaloids, condensed tannins, and coumarins, among others (31).

Tannins are dietary phytochemicals that are responsible for the astringent taste of foods and drinks (32). The presence of tannins can cause browning in fresh foods and processed products, but some tannins also possess the beneficial biological properties mentioned above. The results of this study indicate that 10% substitution of wheat flour with HQCF resulted in bread samples that had higher tannin contents than the 100% wheat bread (Table 3). However, 20%, 30%, and 40% substitution of wheat flour with HQCF resulted in bread samples with tannin concentrations that did not differ significantly from those of the 100% wheat bread.

Alkaloids are classes of plant phytochemicals that have therapeutic and antimicrobial properties (31). As shown in Table 3, the 100% wheat bread contained significantly

higher quantities of alkaloids than the 10% and 40% NRCRI cassava bread samples. However, the alkaloid concentration of the 20% and 30% NRCRI cassava bread samples was not different from that of the 100% wheat bread.

Flavonoids are potent water soluble antioxidants that prevent oxidative cell damage and offer protection against different levels of carcinogenesis (31). One of the most well-known effects of flavonoids on carbohydrate metabolism is the inhibition of α-glucosidase and α-amylase, which are key enzymes responsible for the metabolism of dietary carbohydrates to glucose (33). As shown in Table 3, the 40% NRCRI cassava bread contained significantly higher quantities of flavonoids than the 20% and 30% NRCRI cassava bread samples. However, the flavonoid content of the 40% NRCRI cassava bread was not significantly different from that of the 10% NRCRI cassava bread or the 100% wheat bread.

Cyanogenic glucosides are compounds that yield glucose, hydrogen cyanide, and aldehydes or ketones upon hydrolysis with an acid or enzyme. The lethal dose of cyanide in humans ranges from 50 mg/kg body weight to 300 mg/kg body weight (34,35). Surprisingly, the 10% NRCRI cassava bread and 100% wheat bread had the highest cyanide contents of all of the bread samples investigated. The cyanide contents of the NRCRI cassava bread samples decreased with increasing substitution of wheat flour with cassava flour, except at the 30% HQCF level of substitution (Table 3). One plausible explanation for the higher cyanide contents observed in the 10% NRCRI cassava bread and 100% wheat bread is that the treatment of the grains in these breads with alkaline picrate induced an alkaline hydrolysis that released phenol aglycones from the ester and glycoside compounds within cell-wall polysaccharides (36,37). The released phenol aglycones may have interfered with the results of this assay, thus altering the cyanide concentrations measured in the NRCRI cassava bread samples. Further characterization of the phenolic profile of the bread samples is necessary to validate our claim.

Phytates and oxalates are known to adversely affect mineral bioavailability by forming insoluble salts with zinc, calcium, and iron, thus preventing their utilization.

Table 3. Phytochemical composition of bread samples

Samples	Tannin (%)	Alkaloid (%)	Flavonoid (%)	Cyanide (mg/kg)	Oxalate (mg/100 g)	Phytate (mg/100 g)
Control	0.06±0.01[a]	1.45±0.02[c]	0.75±0.03[b]	10.91±2.89[b]	0.17±0.03[c]	0.28±0.07[c]
10%	0.90±0.14[b]	1.05±0.06[a]	0.85±0.07[bc]	16.35±2.27[c]	0.15±0.02[bc]	0.13±0.03[a]
20%	0.07±0.03[a]	1.15±0.07[ab]	0.60±0.11[a]	5.95±2.57[a]	0.13±0.03[bc]	0.15±0.02[a]
30%	0.08±0.02[a]	1.20±0.02[b]	0.60±0.07[a]	6.80±3.27[a]	0.08±0.03[a]	0.19±0.02[ab]
40%	0.06±0.01[a]	1.05±0.18[a]	0.90±0.02[c]	3.37±1.43[a]	0.11±0.03[ab\]	0.23±0.03[bc]

Values are the mean±SD of three determinations (n=5).
[a-c]Within each column, means with different superscripts are significantly different ($P<0.05$).
Control, 100% wheat bread; 10%~40%, bread containing 10%~40% NRCRI cassava flour.

Table 4. Total viable counts of bacteria and fungi on NA, MA and PDA Plates

Samples	NA	MA	PDA	Bacteria isolated	Fungi isolated
Control	$2.0×10^3$	0	Nil	*Staphylococcus*	Nil
10%	$1.1×10^4$	0	$3×10^3$	*Lactobacillus, Bacillus*	*Aspergillus flavus*
20%	$1.4×10^4$	0	$3×10^3$	*Lactobacillus, Staphylococcus, Bacillus*	*Aspergillus niger*
30%	$1.3×10^4$	0	$2×10^3$	*Lactobacillus, Bacillus*	*Aspergillus flavus*
40%	$9.0×10^3$	0	Nil	*Staphylococcus*	Nil

Values are the means of three determinations.
Control, 100% wheat bread; 10%~40%, bread containing 10%~40% NRCRI cassava flour.

The low amount of these anti-nutrients detected in the bread samples investigated (Table 3) indicates that the minerals that phytates and oxalates typically form complexes are bioavailable in the bread samples tested. Among the bread samples analyzed, there were no significant differences in phytate and oxalate concentrations.

Microbial contamination of food substances can lead to infections that may pose serious health risks to humans. As shown in Table 4, the mean bacteria counts of the bread samples tested ranged from $2.0×10^3$ CFU/mL to $1.4×10^4$ CFU/mL. Among the bread samples tested, the 20% NRCRI cassava bread had the highest bacterial counts, while the 100% wheat bread had the lowest bacterial counts (Table 4).

The fungi counts of the bread samples ranged from 0 CFU/mL to $3×10^3$ CFU/mL (Table 4). All of the NRCRI cassava bread samples analyzed contained higher amounts of bacteria and fungi than the 100% wheat bread. Further microbial analysis indicated the absence of enteric bacteria in all of the bread samples analyzed in this study.

The higher microbial loads of the NRCRI cassava bread samples can be attributed to the higher pH and moisture contents and lower alkaloid contents of the NRCRI cassava bread samples. Our finding of higher microbial loads in NRCRI cassava bread justifies the rationale given by bakers for the slow adoption of HQCF incorporation into wheat flour for bread making.

The microorganisms isolated from the bread samples were: *Lactobacillus* spp., *Staphylococcus, Bacillus, Aspergillus flavus, Penicillium spp.,* and *Aspergillus niger.* Molds are the primary spoilage organisms reported in baked foods, with *Aspergillus, Penicillium,* and *Eurotium* being the most commonly isolated genera. These organisms synthesize toxic secondary metabolites called mycotoxins that have serious health implications (38). *Bacillus* spores are very heat resistant and can survive baking in the interior of bread loaves. *Bacillus* spores that survive baking can germinate and start growing as the bread cools (39).

Although these organisms are potentially pathogenic, their counts (less than 10 CFU units/mL) may be too low to cause any serious health hazard. However, their presence in the NRCRI cassava bread samples tested indicates that precautionary measures should be taken

Table 5. Correlation between total flavonoid content, reducing power, and DPPH antioxidant activity

Parameter	DPPH activity	Flavonoids
Reducing power	0.871**	0.750*
Flavonoids	0.487	

Significant at *$P<0.05$ and **$P<0.01$, respectively.

when using HQCF for the production of cassava/wheat composite bread.

Correlation analyses revealed that there was a significant positive correlation between the reducing powers and the DPPH antioxidant activities of the bread samples ($R^2=0.871$), a significant positive correlation between the reducing powers and the total flavonoid contents of the bread samples ($R^2=0.750$), and a positive correlation between the DPPH antioxidant activities and the total flavonoid contents of the bread samples ($R^2=0.487$) (Table 5).

The significant correlation between the total flavonoid contents and the reducing powers of the bread samples suggests that the flavonoids residing in the lipophilic fractions could be responsible for the reducing powers of the bread samples. This suggests that the use of an extraction media capable of extracting lipophilic antioxidants is necessary for optimal extraction of antioxidants from cassava/wheat composite bread. Furthermore, the higher antioxidant activity of the 100% wheat bread could be attributed to the high levels of flavonoids and phytates present in 100% wheat bread.

Descriptive analysis is one of the most useful tests for sensory profiling. This test uses trained panelists to detect and rate the intensities of the sensory attributes of a product (40). In this study, a panel of 15 trained people evaluated the sensory properties of each bread sample on a 7 point hedonic scale. The results of the descriptive analysis revealed that the color acceptability of the 10% NRCRI cassava bread samples was not significantly different from the color acceptability of the 100% wheat bread (Table 6). The taste of the 100% wheat bread was the most acceptable to the panelists, though it was not significantly different from the 10%, 20%, 30%, or 40% NRCRI cassava bread samples (Table 6). Compared with the other bread samples investigated in this study, the

Table 6. Sensory evaluation of bread samples

Samples	Color	Taste	Flavor	Texture	General acceptability
Control	4.22±0.16[ab]	4.39±0.24[a]	4.78±0.16[c]	4.11±0.31[a]	4.84±0.08[b]
10%	4.17±0.08[ab]	3.73±0.08[a]	3.45±0.16[a]	4.23±0.78[a]	4.22±0.16[ab]
20%	3.95±0.39[ab]	3.78±0.15[a]	3.45±0.47[a]	4.16±0.40[a]	4.28±0.40[b]
30%	3.72±0.71[a]	3.73±0.23[a]	3.45±0.16[a]	4.00±0.47[a]	3.62±0.07[a]
40%	3.67±0.16[a]	3.95±0.87[a]	3.89±0.47[ab]	3.89±0.16[a]	3.62±0.07[a]

Values are the mean±SD of three determinations (n=5).
[a-c]Within each column, means with different superscripts are significantly different ($P<0.05$).
Control, 100% wheat bread; 10%~40%, bread containing 10%~40% NRCRI cassava flour.
Scoring system: 1=Very poor; 2=Poor; 3=Fair; 4=Average; 5=Good; 6=Very good; 7=Excellent.

flavor of the 100% wheat bread was the most acceptable to the sensory panelists (Table 6). While the difference was not significant, the 10% NRCRI cassava bread had a better texture than the other bread samples investigated. The 100% wheat bread was the most generally acceptable to the sensory panelists. However, the general acceptability of the 100% wheat bread was not significantly different from that of the 10% or 20% NRCRI cassava bread samples (Table 6). The results obtained from this study show that substitution of wheat flour with up to 20% HQCF can yield bread samples with a general acceptability that is comparable to 100% wheat bread.

CONCLUSION

This study showed that substitution of wheat flour with varying levels of HQCF (i.e., 10%, 20%, 30%, and 40%) decreased the mineral and protein contents of bread samples in a dose dependent manner. The crude fiber contents of the 10%, 30%, and 40% NRCRI cassava breads, as well as the 100% wheat bread, were below the detection level, while the crude fiber content of the 20% cassava/composite bread was very low. Substitution of wheat flour with up to 20% HQCF yielded bread samples with a general acceptability that was comparable to that of 100% wheat bread. The incorporation of 10%, 20%, and 40% HQCF into bread decreased the lipid content of the dough. However, lipid content was increased with 30% cassava substitution. The significant correlation between the total flavonoid contents and the reducing powers of the bread samples tested suggests that the flavonoids residing in the lipophilic fractions of the bread could be responsible for the reductive capacities of the bread samples.

The higher microbial load of the NRCRI cassava bread samples is attributed to the higher pH and moisture contents and lower alkaloid contents of the NRCRI cassava breads compared with the 100% wheat bread. This elevation in microbial load supports previous reports by bakers that bread samples containing HQCF deteriorate faster than those produced from 100% wheat flour. Thus, we recommend that bread samples produced with cassava/wheat composite flour be consumed within a short time to avoid microbial deterioration.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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