



Assessment of Nutritional Compositions, Functional Properties, Cyanogenic Potential and Carotenoids Retention in Dark Processed Pro-Vitamin A-Enriched Cassava/Wheat Composite Flour

Josephine Odunayo Ilade¹, Adekunle Isiaka Amoo^{1*}, Ganiyu Oboh², Stephen Adeniyi Adefegha²

¹Chemistry Department, Federal University of Technology, P. M. B 704, Akure, Ondo State, Nigeria.

²Department of Biochemistry, Federal University of Technology, P. M. B 704, Akure, Ondo State, Nigeria.

*Corresponding author email: adisamoo@yahoo.co.uk; Tel: +2348034016068

Abstract

Recent studies unraveled the roles of vitamin A-deficiency in the integrity of pancreatic β -cells and diabetes pathogenesis. Cassava is a cheap and largely cultivated staple crop in Nigeria. The flour can be incorporated into wide varieties of food products and could serve as a medium for micronutrient deficiency intervention. This study investigated the nutritional qualities, Hydrogen cyanide content, and carotenoid retention in pro-vitamin A-enriched cassava and wheat composite flour. The yellow cassava tubers (*IBA154810*) was obtained from the International Institute of Tropical Agriculture (IITA) Ibadan Oyo State Nigeria and processed into flour. Each flour blends were produced by mixing pro-vitamin A-enriched cassava flour (ACF) with wheat flour (WF) in ratio: 100%WF(control), 20% ACF:80% WF, 40% ACF:60% WF, 60% ACF:40% WF, 80% ACF:20% WF, 100% ACF. The water absorption capacity, oil absorption capacity, bulk density, foaming capacity and foaming stability of the samples ranged from (136.40 – 161.66)%, (125.40 – 153.66)g/ml, (0.75–0.81)%, (15.67–38.26)%, (3.98–19.57)% and (67.57–85.68) $^{\circ}$ C respectively. Total β -carotenoids, Retinol Activity Equivalent, ascorbic acid and fibre ranged from (75.6–1256 μ g/100g), (20.43 – 432.27 μ g/100g), (9.33–48.47 μ g/100g), and (1.61–2.26g/100g) respectively. Total β -carotenoids, ascorbic acid, fibre, K, Ca, and Mg content of composite samples increases with cassava flour substitution. Ca is the most abundant mineral followed by K. The low cyanide content (0.08–2.23mg/kg) could guarantee sustainable production plan by minimizing energy and time utilised for detoxification process and prevent environmental contamination. The data revealed that ACF/WF composite samples had higher nutritional qualities compared to 100% WF (control) and could help combat the problem of vitamin A-deficiency and its associated diseases in Nigeria and other developing cassava-consuming countries.

Keywords: Cassava, Vitamin A-Deficiency, Composite Flour, Local Productivity, Food formulations

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is a common root crop that provides food for over 500 million people in the world (Chisenga *et al.*, 2019 and Bouniol *et al.*, 2021). It is one of the most important food crops consumed extensively as a major staple food in Africa with Nigeria, Thailand, Brazil, Indonesia and Congo Democratic Republic being its largest producers (Rome 2020, Droppelmann *et al.*, 2018). Cassava flour is a major raw material in several food industry (Nilusha *et al.*, 2021), however except for carbohydrate, cassava is not a good source of protein and fats but when consumed with other foods makes products of high nutritional quality (Laswai *et al.*, 2017). Many studies have shown low occurrence of diabetes in Africans who consume cassava regularly. Most

African countries are facing food and nutritional insecurity which manifests itself in the form of low intake of essential nutrients in the diets of people.

Cassava has been distinguished as an appropriate crop for micronutrient deficiency intervention in Africa (Ilona *et al.*, 2017). Breeding efforts have brought about the development of new cassava variants possessing desirable traits such as increased root, flour, and starch yield, reduced toxicity, reduced pest/disease susceptibility and improved nutrient contents. Prominent among those breeding efforts is the development of yellow-flesh cassava variants, which has been genetically modified to accumulate high levels of pro-vitamin A carotenoids (Oluwatoyin *et al.*, 2018). Vitamin A deficiency

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(VAD) is a serious health crisis among children and women in sub-Saharan Africa (Arlappa *et al.*, 2011; Bowley, 2008). Statistical report stated that one out of every three children under five and one-quarter of every expectant mothers in Nigeria are vitamin A deficient (Maziya-Dixon *et al.*, 2006). Previous studies revealed the role of vitamin A deficiency as a contributing factor to poor glucose metabolism and progression of type 2 diabetes (Khaliq *et al.*, 2022; Zhou *et al.*, 2021) and other related diseases such as macular degeneration and cataracts (Udensi *et al.*, 2022). Several intervention strategies have been advanced to address some of the devastating consequences of VAD in children (Oluba, 2017). Antioxidant (pro-vitamin A carotenoids, and vitamin C) – rich foods have been known protect against certain chronic diseases, including diabetes, heart disease and cancer (Adefegha, 2018), through the elimination of ROS (Caturano *et al.*, 2023).

More importantly, for most developing sub-Saharan African Countries where the growth of wheat is limited, the high foreign exchange cost on wheat importation is adversely affecting the economy. The high cost of wheat importation and wheat products coupled with its high glycemic index necessitated the search for a more available, cheaper and healthier alternative. As a result, this research work was aimed to develop a better processing method for pro-Vitamin A-enriched Cassava/wheat composite flour to enhance better β -carotenoids retention and assessment of its nutritional qualities and suitability as a better alternative substitute for wheat flour. This will help address the problem of vitamin A deficiency,

create awareness to embrace local productivity and dwindle unnecessary wheat importation especially in Nigeria.

2. Material and Methods

2.1 Sample Collection

Matured yellow vitamin A enriched cassava tubers (IBA154810), a year old was bought from International Institute of Tropical Agriculture (IITA) Ibadan Oyo State Nigeria. The new cassava variant was authenticated with registration number (0110) at the herbarium unit of the Federal University of Technology Akure, Ondo State Nigeria.

2.2 Preparation of Vitamin A-Enriched Cassava Flour

The cassava tubers were processed into flour according to the method of Agbemafle (2019) but carried out in dark laboratory condition. As shown in Figure 1, the freshly harvested cassava tubers were quickly transported to the food laboratory using dark sacks. It was thoroughly washed, peeled using knives, rinsed and shredded into long, thin strips using a manual vegetable shredder. It was then dried immediately in hydrator at 60°C for 5 hours, milled into flour. The ACF produced and WF was separately passed through a 500 μ m sieve. As presented in Table 1, each flour blends were produced by mixing pro-vitamin A-enriched cassava flour (ACF) with wheat flour (WF) in the following ratio: 100%WF(control), 20% ACF:80%WF, 40%ACF:60%WF, 60%ACF:40%WF, 80%ACF:20%WF, 100% ACF. The flour samples were packaged in black airtight containers prior to analysis.

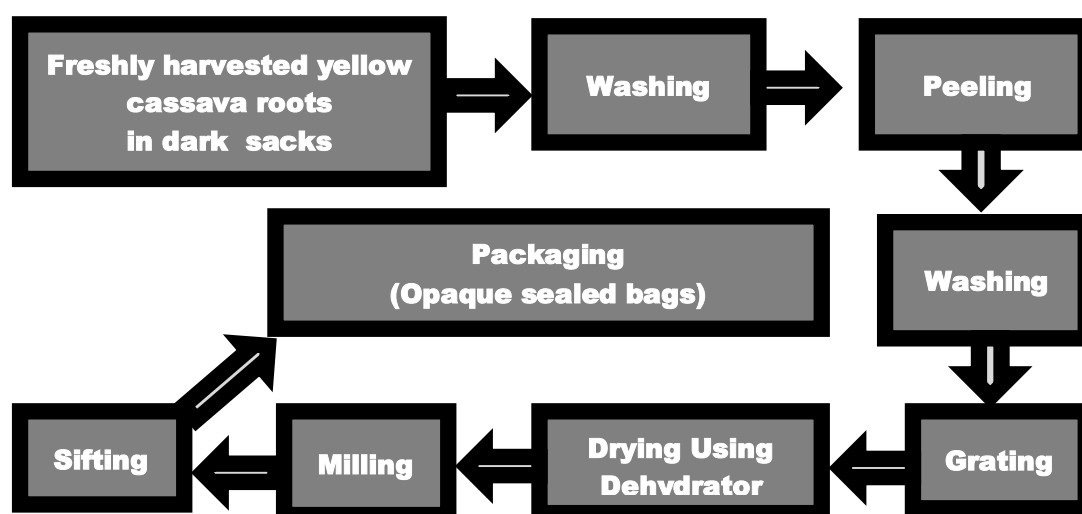


Figure 1: Flow chart of pro-vitamin A-Enriched cassava dark processing into flour

Table 1: Ratio of composite flour formulation

Sample	Ratio of Flour Blends	
	ACF	WF
100WF (control)	0	100
20ACF/80WF	20	80
40ACF/60WF	40	60
60ACF/40WF	60	40
80ACF/20WF	80	20
100ACF	100	0

Keys: 100WF (control) = 100% wheat flour, 20ACF/80WF = 20% pro-vitamin A-enriched cassava flour + 80% wheat flour 40ACF/60WF = 40% pro-vitamin A-enriched cassava flour + 60% wheat flour 60ACF/40WF = 60% pro-vitamin A-enriched cassava flour + 40% wheat flour, 80ACF/20WF = 80% pro-vitamin A-enriched cassava flour + 20% wheat flour, 100ACF = 100% pro-vitamin A-enriched cassava flour

The moisture, ash, fat, crude fibre, protein and carbohydrate content were determined using the methods of AOAC (2012). The Energy values were calculated using Atwater conversion factors by Nguyen *et al.*, (2007). The dry matter content were obtained from moisture content analysis and expressed as percentage by the relation: % DM = (100 - % moisture) Adubofuor *et al.*, (2014).

2.4 Determination of Mineral Composition

Mineral Compositions such as Ca, Mg, Na, Fe, K and Zn were determined using Atomic Absorption Spectrophotometer (Buck Scientific, Model 235) (AOAC, 2012).

2.5 Functional Properties

2.5.1 Gelatinization temperature

This was determined by adding 10 ml of distilled water to 1g of flour. The mixture was incubated in a water bath until a solid gel was formed. The temperature, which plays a role in the gel formation, was measured as the gelatinization temperature (Chandra *et al.*, 2014).

2.5.2 Foaming capacity (FC) and Foam stability (FS)

These were determined by the method described by Sze-Tao and Sathe (2000) with certain modification. 1g of each sample was isolated in 50 cm³ of distilled water. The solution was stirred at a speed of 160 rpm for 10 min. The blend was immediately transferred into a 100cm³ graduated cylinder. The volumes were recorded before and after stirring. For the determination of foam stability, foam volume changes in the graduated cylinder were recorded at 25 min of storage.

$$FC (\%) = \frac{(\text{Volume after whipping} - \text{Volume before whipping})\text{ml}}{(\text{Volume before whipping})\text{ml}} \times 100$$

$$FS (\%) = \frac{(\text{Volume after standing} - \text{Volume before whipping})\text{ml}}{(\text{Volume before whipping})\text{ml}} \times 100$$

2.5.3 Bulk Density (BD)

A weighed sample was transferred into 10 cm³ graduated cylinder. The bottom of the cylinder was gently tapped on a laboratory bench for several times until no further diminution of the sample level was observed after it was filled up to the calibrated mark. Bulk density is expressed as:
Bulk density (g/cm³) = Weight of sample (g) / Vol. of sample (cm³)

2.5.4 Oil absorption capacity (OAC)

One (1 g) of each sample was weighed into 20cm³ pre-weighed centrifuge tubes and thoroughly mixed with 5cm³ vegetable oil. The emulsion was kept for 25 min at room temperature and centrifuged for 15 min. the supernatant was carefully removed, and the tube was reweighed Lin and Zayas (1987)

$$OAC (\%) = \frac{T_2 - T_1}{T_0} \times 100$$

Where: T₀ is the weight of sample, T₁ is the weight of tube plus sample, and T₂ is the weight of tube plus sediment.

2.5.5 Water Absorption Capacity (WAC)

One gram (1g) of each flour sample was weighed into 20 cm³ pre-weighed centrifuge tube. 10 cm³ of distilled water was added in to the tube under continuous stirring with a glass rod at room temperature for 25 min and centrifuged for 20 min. The percentage WAC was calculated by:

$$WAC (\%) = \frac{W_2 - W_1}{W_0} \times 100$$

Where W₀ is the weight of sample, W₁ is the weight of tube plus sample, and W₂ is the weight of tube plus sediment. (Rodriguez-Ambriz *et al.*, 2005)

2.6 Ascorbic acid analysis

Five gram (5g) of bread crumb was soaked in methanol (50mL), kept in the dark for 3 days and filtered. The extract obtained was then evaporated using a rotary evaporator to obtain a concentrated extract in each solvent. 10 mg ascorbic acid (AA) was dissolved with distilled water in a 100 ml volumetric flask and adjusted to the mark, shaken to obtain a solution of 100 ppm ascorbic acid. From 100ppm stock solution, using a micropipette, volume 400 µl, 600 µl, 800 µl, 1 ml and 2 ml was taken and made up to the mark in a 10ml volumetric flask to obtain AA concentration of 4, 6, 8, 10 and 12 ppm respectively for the calibration curve. 100 mg of the bread methanolic extract was put into a 100 mL volumetric flask and made up to the mark with distil water. Measurement of vitamin C levels in bread extracts using the UV-Vis spectrophotometry method was carried out using distilled water as a blank and ascorbic acid as a standard solution. The absorbance of standard and sample was read at 450nm (Novia Annisa *et al.*, 2023).

2.7 Total β-Carotenoids Determination

The total β-carotenoids were determined using the spectrophotometry method of harvestplus handbook for carotenoid analysis by Delia *et al.* (2004). Five of the pro-vitamin A enriched cassava tubers sample were taken at random, washed, peeled, and quartered longitudinally from the stem end to the root end. Two opposite sections from each root were taken and combined. The large pieces were cut into smaller pieces and rapidly homogenized in a food processor. 15g of the homogeneous representative raw sample/ 15g of each flour sample was weighed and transferred to a mortar. 3g of Hyflosupercel (celite) was added to the sample and the entire mixture was ground with 50ml of cold acetone until residue was devoid of colour and suction filtered using a Buchner funnel with filter paper. 20ml of petroleum ether (PE) was poured into 500ml separating funnel. The acetone extracts were added. 300ml of distilled water was slowly added along the walls of funnel to avoid emulsion formation. After phase separation, the aqueous phase was discarded. The PE phase was rinsed four times with 200ml distilled water. The PE extract was then filter through a funnel containing 15g anhydrous sodium sulphate, concentrated and the absorbance read at 450nm. The Retinol Activity Equivalents was carried out using the conversion factor stated in Awoyale *et al.*, (2018)

$$\text{Total carotenoids content } (\mu\text{g/g}) = \frac{A \times \text{Vol. (mL)} \times 10^4}{A_{1\text{cm}}^{1\%} \times \text{sample weight (g)}}$$

Where A = absorbance; Vol. = total volume of extract (25mL); A_{1cm}^{1%} = Absorbance coefficient of β-carotene in PE (2592)

2.8 Determination of Cyanide Content

Four gram (4g) of each flour sample was weighed and soaked in a mixture of 40ml distilled water and 2 ml orthophosphoric acids. The mixture was thoroughly mixed, covered and allowed to stand overnight at room temperature to release the bonded hydrocyanic acid. The resulting solution was distilled. 45ml of the distillate was collected into a receiving volumetric

flask containing 4ml of distilled water and 0.1g sodium hydroxide pellets. It was made up to 50ml with distilled water. 20ml was pipetted into a conical flask. 1.6ml of 5% potassium iodide solution was added and titrated against 0.01M Ag(NO₃) solution to a faint permanent turbid end point. The procedure was repeated for blank by replacing the samples with distilled water (AOAC 2012). The cyanide content in mg/kg was calculated using the formula below:

$$\text{HCN (mg/kg)} = 13.5 \frac{(V_0 - V_1)}{M}$$

V₀=Titre Value of Sample, V₁= Titre Value of Blank, M = Mass of Sample

2.9 Statistical Analysis

The data obtained from the procedures was collated and analyzed using Statistical Package of Social Science (SPSS) version 21.0. The means obtained was separated using Duncan's Multiple Range Tests at 5% level of probability

3. Results and Discussion

3.1 Proximate analysis of pro-vitamin A- enriched cassava / wheat composite flour samples

The proximate compositions of the ACF/WF composite samples are presented in Table 2. The moisture content is a useful parameter that determines the extent of storage and shelf life of cassava flour. Low value is required for storage stability as values above 12% have been known to trigger microbial growth and spoilage (Oluranti *et al.*, 2022 and Robert, 2019). The moisture content of the composite samples ranged from (9.11 – 9.81%) with significant (p<0.05) differences, though a little higher compared to values reported by (Gregory *et al.*, 2023 and Nilusha *et al.*, 2021) for different cassava cultivars flours. These differences could be attributed to differences in the nature of soil, processing method, variety and season. The 100WF(control) had the highest value of (9.81± 0.08%) while 100ACF sample had the least value (9.11 ± 00.5%). The results revealed that the moisture content decreases with increasing addition of 100ACF which suggests that 100ACF had lower moisture content compared to the 100WF. However, moisture content of all the samples was low and would help prevent microbial growth and extends the shelf life of the flour samples.

The Ash, fibre, carbohydrate, and dry matter content of the bread samples differed significantly (p< 0.05) and ranged from (2.23 –

3.21%), (1.61 – 2.26%), (71.73 – 83.77%), and (90.19 – 90.88 %) respectively. These four parameters were observed to increase as the proportion of ACF increases. More also, the control sample had the lowest values. This implies that only the ACF contributed to the increasing parameters of the samples. The ash content of a food is an expression of its mineral compositions (Robert, 2019). As shown in Table 2, flour samples with higher ash content could reflect higher mineral content than the others. Dietary fiber is essential due to its ability to ease bowel movement, and prevention of several gastrointestinal diseases (Satinder *et al.*, 2011). Aside the high proportion of starch in cassava flour (Ayetigbo *et al.*, 2018), its high ash and fibre content compared to wheat flour might have also contributed to the increasing carbohydrate and dry matter content of the composite samples.

The fat and protein content of the ACF/WF composite samples ranged from (0.55 – 2.12 %) and (1.13 – 12.49 %) with significant difference (p<0.05). These two parameters decrease with increasing incorporation of ACF. This trend was also reported by Ayele *et al.*, (2017) for cassava based products. The highest protein value recorded for 100% wheat flour could be attributed to its gluten content which is absent in the cassava flour. High gluten foods have been known to cause an autoimmune disorder and celiac disease in some individuals (Olatunde *et al.*, 2020). The decrease in the protein content of the composite flour samples with increasing ACF inclusion may not be desirable due to its contribution to cellular growth and development. However, the protein content of the ACF/WF and 100ACF samples could be augmented with addition of preferred protein-rich ingredient during food formulation.

The decreasing fat level of the flour samples is noteworthy as foods with low fat content are usually recommended for diabetic patients and persons with high risk of developing cardiovascular diseases. The carbohydrate contents of composite flour samples ranged from (71.73 – 83.77%). Looking at the results in Table 2, a similar trend of increasing carbohydrate content with the incorporation of larger proportion of cassava flours have been reported in previous studies (Eddy *et al.*, 2007) owing to high starch of cassava flour. The energy content ranged from (343.98 – 356.09 kcal/100g). The highest energy content recorded for 20ACF/80WF followed by 100WF might be attributed to higher fat and protein content of wheat flour compared to the cassava flour.

Table 2: Proximate analysis of pro-vitamin A- enriched cassava/wheat composite flour samples

Sample	%Moisture	%Ash	%Fat	%Fibre	%Protein	%CHO	DM	Energy (kcal/100g)
100WF (control)	9.81 ^a ± 0.08	2.23 ^c ±0.02	2.12 ^a ±0.01	1.61 ^d ±0.08	12.49 ^a ±0.02	71.73 ^f ±0.11	90.19 ^d ±0.08	356.00 ^a ±0.60
20ACF/80WF	9.64 ^b ± 0.05	2.42 ^c ±0.03	2.03 ^{ab} ±0.06	1.65 ^d ±0.05	10.02 ^b ±0.07	74.42 ^e ±0.35	90.34 ^c ±0.04	356.09 ^a ±1.73
40ACF/60WF	9.41 ^c ± 0.04	2.21 ^c ±0.06	1.98 ^b ±0.02	1.96 ^c ±0.05	9.27 ^c ±0.06	75.11 ^d ±0.07	90.60 ^b ±0.01	355.42 ^a ±0.24
60ACF/40WF	9.41 ^c ± 0.07	2.71 ^b ±0.02	1.03 ^c ±0.06	1.88 ^c ±0.02	5.64 ^d ±0.09	79.34 ^e ±0.17	90.59 ^b ±0.07	349.21 ^b ±0.39
80ACF/20WF	9.19 ^d ± 0.02	2.70 ^b ±0.29	0.67 ^d ±0.04	2.09 ^b ±0.11	2.62 ^e ±0.04	82.69 ^b ±0.39	90.81 ^a ±0.02	347.24 ^c ±1.39
100ACF	9.11 ^d ± 00.5	3.21 ^a ±0.05	0.55 ^e ±0.08	2.26 ^a ±0.09	1.13 ^f ±0.01	83.77 ^a ±0.09	90.88 ^a ±0.05	343.98 ^d ±0.55

Note: The Mean ± SD values in the same columns with varied superscript are significantly different (p < 0.05), n = 3.

Keys: 100WF (control)= 100% wheat flour, 20ACF/80WF = 20% pro-vitamin A-enriched cassava flour + 80% wheat flour 40ACF/60WF = 40% pro-vitamin A-enriched cassava flour + 60% wheat flour 60ACF/40WF = 60% pro-vitamin A-enriched cassava flour + 40% wheat flour, 80ACF/20WF = 80% pro-vitamin A-enriched cassava flour + 20% wheat flour, 100ACF = 100% pro-vitamin A-enriched cassava flour

3.2 The mineral composition of the pro-vitamin A-enriched cassava/wheat composite flour samples

The minerals compositions of the composite flour are shown in Table 3. The data revealed that the concentration of K, Ca and Mg increases with increasing ACF substitution. The

results show calcium is the most abundant mineral in all the flour samples and highest in the added ACF which agrees with the findings of Oluwaniyi and Oladino (2017). The produced composite flour samples would serve as a good source of dietary calcium supplement which could help avoid

osteoporosis and rickets in consumers due to calcium deficiency. The second most abundant mineral in the composite flour samples is potassium. It has been known to lower high blood pressure, regulates fluid balance and reduces the chances of developing cardiovascular diseases (Wang *et al.*, 2022). The sodium, potassium (Na:K) ratio results obtained for all the experimental flour samples were significantly ($P < 0.05$) lower than 1.00 except the 100WF (control). According to (FAO/WHO/UNU, 2007), Na:K

values > 1.00 recorded for ACF/WF composite samples and 100ACF were regarded as safe food if consumed by the hypertensive individuals (Olugbuyi *et al.*, 2023). The values of Na, Fe and Zn were significantly different ($P < 0.05$) for all samples with the 100WF (control) sample having the highest concentrations of 41.20ppm and 2.46ppm for Na and Fe respectively. The highest Zn content of 4.58ppm was observed in the 20ACF/80WF.

Table 3: Mineral composition of pro-vitamin A- enriched cassava/wheat composite flour samples (ppm)

Sample	Na	K	Ca	Mg	Fe	Zn	Na : K
100WF (control)	41.20 ^a ± 1.76	23.75 ^c ± 1.76	145.00 ^d ± 10.60	23.75 ^c ± 1.76	2.46 ^a ± 0.12	3.90 ^b ± 0.02	1.74
20ACF/80WF	17.50 ^{bc} ± 3.53	55.00 ^c ± 3.35	181.25 ^d ± 3.50	30.00 ^b ± 0.00	0.77 ^c ± 0.02	4.58 ^a ± 0.03	0.31
40ACF/60WF	8.75 ^d ± 1.76	72.50 ^b ± 0.06 ^b	248.75 ^c ± 22.90	28.75 ^b ± 1.76	0.86 ^c ± 0.07	2.58 ^d ± 0.01	0.12
60ACF/40WF	10.00 ^d ± 3.53	98.75 ^{ab} ± 19.44	321.25 ^b ± 26.51	31.25 ^b ± 1.76	1.25 ^b ± 0.02	2.84 ^c ± 0.02	0.10
80ACF/20WF	13.75 ^{cd} ± 1.76	148.50 ^a ± 47.37	363.75 ^{ab} ± 22.98	40.00 ^a ± 0.00	0.51 ^d ± 0.06	1.69 ^e ± 0.02	0.09
100ACF	20.00 ^b ± 0.00	140.00 ^a ± 10.60	372.50 ^a ± 7.07	37.50 ^a ± 0.00	0.86 ^c ± 0.02	1.14 ^f ± 0.00	0.14

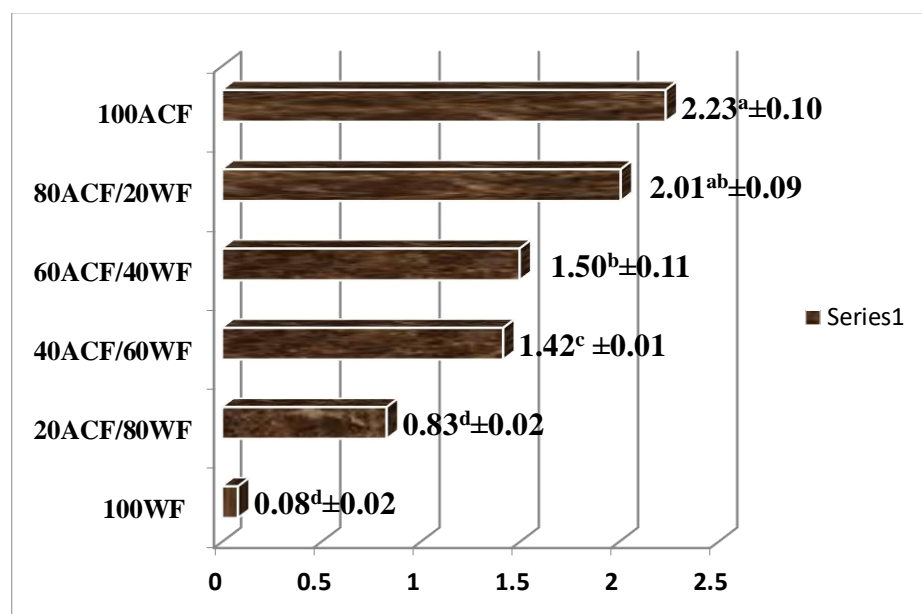
Note: The Mean ± SD values in the same columns with varied superscript are significantly different ($p < 0.05$), $n = 3$.

Keys: 100WF (control) = 100% wheat flour, 20ACF/80WF = 20% pro-vitamin A-enriched cassava flour + 80% wheat flour 40ACF/60WF = 40% pro-vitamin A-enriched cassava flour + 60% wheat flour 60ACF/40WF = 60% pro-vitamin A-enriched cassava flour + 40% wheat flour, 80ACF/20WF = 80% pro-vitamin A-enriched cassava flour + 20% wheat flour, 100ACF = 100% pro-vitamin A-enriched cassava flour

3.3 Hydrogen Cyanogenic Content of the Pro-Vitamin A-enriched Cassava-Wheat Flour Blend Samples

Cyanide is the most toxic factor restricting the direct consumption of cassava tubers and leaves (Adugna 2019). Despite the fact that cassava roots are rich in carbohydrates, fiber and some essential minerals and vitamins, its high cyanogenic glycosides is a major challenge which has to be thoroughly detoxified during processing (Prathibha *et al.*, 1995). Recently, breeding efforts have led to the development of cassava variants with desirable traits such as improved yield, reduced toxicity, reduced pest/disease susceptibility and improved nutrient contents (Oluwatoyin *et al.*, 2018). Most biofortified yellow-fleshed variants with increased pro-vitamin A carotenoids has low cyanide content (GU *et al.*, (2013). The prepared yellow cassava tubers was used to feed

school children in some region of Africa where many children die as a result of micronutrient deficiency to partially meet the recommended vitamin A daily requirements (UNICEF, 2005 and Talsma, 2014). The cyanide content the formulated ACF/WF composite flour samples, ranging from (0.08±0.03 – 2.23±0.10)mg/kg are at levels within the FAO/WHO (1991) recommendations, < 10 mg/kg dry matter for safe cassava products. Most bio-fortified cassava variants contains significant important vitamins which are sensitive to light, heat and leaches into water during processing (Favier, 1977). The low level reported in this study as shown in Figure 2 would prevent the fear of acute human toxicity; reduce time and extremely rigorous processing methods that can affect its nutritional value through modifications and losses of essential nutrients.



Note: Bars with Mean ± SD values in the same with varied superscript are significantly different ($p < 0.05$), $n = 3$.

Keys: 100WF (control) = 100% wheat flour, 20ACF/80WF = 20% pro-vitamin A-enriched cassava flour + 80% wheat flour 40ACF/60WF = 40% pro-vitamin A-enriched cassava flour + 60% wheat flour 60ACF/40WF = 60% pro-vitamin A-enriched cassava flour + 40% wheat flour, 80ACF/20WF = 80% pro-vitamin A-enriched cassava flour + 20% wheat flour, 100ACF = 100% pro-vitamin A-enriched cassava flour

3.4 Total beta-carotenoids and ascorbic acid content

Carotenoids have drawn significant attention recently due to their antioxidant activity and their important roles in protection against cancer and cardiovascular diseases (Udensi *et al.*, 2022). Oxidative stress has been known to be a critical factor contributing largely to the pathogenesis and progression of diabetes and cancer (Caturano *et al.*, 2023 and Vilchis-Landeros *et al.*, 2024). The action of carotenoids against these life threatening diseases has been attributed to their antioxidant activity, specifically to their ability to quench singlet oxygen and interact with free radicals (Delia *et al.*, 2011). However, their ability to absorb light in the visible region as a result of the long chain conjugated double bond in their structure quickly make them prone to photo-oxidative degradation (Britton, 1996) and great loss during home or commercial food processing. Most cassava post harvest processing and storage methods further expose the carotenoids to degradation thereby minimizing their bioavailability and health benefits.

The total β -carotenoids, the corresponding Retinol Activity Equivalents and ascorbic acid content of the flour samples ranged from $(75.6 \pm 0.06 - 1256.40 \pm 0.05) \mu\text{g}/100\text{g}$, $(20.43 - 432.27 \mu\text{g}/100\text{g})$ and $(9.33 \pm 0.12 - 48.47 \pm 0.21) \mu\text{g}/100\text{g}$ respectively. The higher percentage retention values recorded in this study as shown in Table 4 compared to those reported by Awoyale *et al.*, (2018) could be likely due to limited exposure to light from the onset of harvesting, during processing, degree/duration of heat treatment, nature of packaging materials used, differences in analytical methods as well as the newly released yellow cassava variant (IBA154810) with higher carotenoids used for the study. Oven-drying between $50^\circ - 60^\circ\text{C}$ has been proven to maintain the best retention with proper handling compared to the fresh sample than sun drying (vimala *et al.*, 2011). From a nutritional perspective, the formulated ACF/WF flour samples may help address the problem of vitamin A deficiency and its associated diseases (Ospina *et al.*, 2023 and Khalique *et al.*, 2022), especially among sub-Saharan Africans where cassava is largely cultivated and consumed as a major staple food.

Table 4: The total beta-carotenoids, Retinol Activity Equivalents (RAE) and Ascorbic acid content of pro-vitamin A- enriched cassava/ wheat composite flour samples

Sample	TBC $\mu\text{g}/100\text{g}$ (Flour)	RAE ($\mu\text{g}/100\text{g}$)	Retention (%)	Ascorbic acid ($\mu\text{g}/100\text{g}$)
100WF	$75.6^e \pm 0.06$	20.43	-	$9.33^c \pm 0.12$
20ACF/80WF	$493.37^f \pm 0.11$	133.34	30.85	$14.36^c \pm 0.01$
40ACF/60WF	$769.63^e \pm 0.04$	207.47	47.99	$29.72^b \pm 0.85$
60ACF/40WF	$880.90^d \pm 0.11$	238.08	55.08	$33.57^b \pm 0.05$
80ACF/20WF	$976.57^c \pm 0.07$	263.94	61.05	$38.52^{ab} \pm 0.04$
100ACF	$1256.40^b \pm 0.05$	339.57	78.55	$48.47^a \pm 0.21$
Fresh Cassava Tuber	$1599.40a \pm 0.01$	432.27		

Note: The Mean \pm SD values in the same columns with varied superscript are significantly different ($p < 0.05$), $n = 3$.

Keys: 100WF (control)= 100% wheat flour, 20ACF/80WF = 20% pro-vitamin A-enriched cassava flour + 80% wheat flour 40ACF/60WF = 40% pro-vitamin A-enriched cassava flour + 60% wheat flour 60ACF/40WF = 60% pro-vitamin A-enriched cassava flour + 40% wheat flour, 80ACF/20WF = 80% pro-vitamin A-enriched cassava flour + 20% wheat flour, 100ACF = 100% pro-vitamin A-enriched cassava flour

3.5 Functional properties of pro-vitamin A-enriched cassava/wheat composite flour samples

There were significant differences ($p < 0.05$) in the water absorption capacity of the flour samples as presented in Table 5. The water absorption capacities ranged between $136.40 \pm 0.10 - 161.66 \pm 0.15$ %. The increasing WAC is an indication that the ACF starch has higher ability to associate with water than the WF. Flours with the ability to absorb water and swell have beneficial applications in dough manipulations during food processing (Kinsella, 1976). Results obtained showed that there were significant differences ($p < 0.05$) in the oil absorption capacities of all the ACF/WF composite samples. The added 100ACF recorded the highest oil absorption capacity with a value of 156.23 ± 0.05 % followed by 80ACF/20WF with a value of 153.66 ± 0.58 % while 40ACF/60WF had the lowest oil absorption capacity of 125.40 ± 0.10 %. The Oil absorption capacity is an important factor in food formulations as oil improves the mouth feel of foods, aids flavour retention, and extends the shelf life particularly food products (Aremu *et al.*, 2007).

The bulk density of an agricultural commodity is an important factor to consider in commerce as it provides information towards efficient and cost effective storage, packaging and timely delivery of goods to consumers. It has also been considered as measure of the heaviness of flour according to Adejuyitan *et al.*, (2009). The bulk densities of the flours

ranged from $(0.75 \pm 0.00 - 0.81 \pm 0.01)$. There is no significant difference ($p < 0.05$) in bulk density from 40 to 80% ACF substitution. However, the same and highest values recorded for 80ACF/20WF and 100ACF samples indicate that more quantity of the samples can be packaged within a constant volume thereby reducing the cost of packaging materials and transportation Shittu *et al.*, (2005).

The foaming capacity (FC) and stability (FS) ranged from $(15.67 \pm 0.03 - 38.26 \pm 0.02)$ % and $(3.98 \pm 0.04 - 19.57 \pm 0.05)$ % respectively. 100WF(control) records the highest foaming capacity and stability with the values of 38.26 ± 0.02 % and 19.57 ± 0.05 % while 100ACF sample had the lowest values for the two parameters as shown in Table 5. The decreasing foaming capacity and stability of the composite flour samples is due to low protein content of cassava which is a good foaming agent. The foaming capacity of a protein refers to the amount of interfacial area that can be created by the protein and foam stability refers to the ability of protein to stabilize against gravitational and mechanical stresses (Fennema, 1996). The capacity of proteins to form stable foams with gas by forming impervious protein films is an important property in bakery and confectionaries and is used to improve consistency, texture, and appearance of foods. The decreasing protein content and foaming parameters of the composite flour samples may be augmented by the addition of protein-rich ingredients such as egg, protein isolates/concentrates and legumes flour during food formulation.

Table 5: Functional properties of pro-vitamin A- enriched cassava/wheat composite flour samples

Sample	WAC (%)	OAC (%)	BD(g/ml)	FC (%)	FS (%)	GT (°C)
100WF	136.40 ^f ± 0.10	127.43 ^e ± 0.60	0.75 ^b ± 0.00	38.26 ^a ± 0.02	19.57 ± 0.05	67.57 ^e ± 1.15
20ACF/80WF	138.47 ^e ± 0.11	133.83 ^d ± 0.11	0.76 ^b ± 0.04	29.45 ^b ± 0.04	15.66 ± 0.03	70.22 ^d ± 0.03
40ACF/60WF	138.77 ^d ± 0.05	125.40 ^f ± 0.10	0.78 ^a ± 0.02	23.56 ^c ± 0.07	11.72 ± 0.07	74.90 ^c ± 0.08
60ACF/40WF	145.16 ^c ± 0.05	145.67 ^c ± 0.15	0.80 ^a ± 0.09	21.62 ^d ± 0.08	11.73 ± 0.04	76.27 ^b ± 0.06
80ACF/20WF	157.40 ^b ± 0.10	153.66 ^b ± 0.58	0.81 ^a ± 0.01	17.65 ^e ± 0.01	7.83 ± 0.02	85.13 ^a ± 0.11
100ACF	161.66 ^a ± 0.15	156.23 ^a ± 0.05	0.81 ^a ± 0.00	15.67 ^f ± 0.03	3.98 ± 0.04	85.68 ^a ± 0.01

4. Conclusion

The study on the assessment of nutritional qualities, functional properties, cyanogenic potential and carotenoids retention in pro-vitamin A-enriched cassava/wheat composite flour revealed that increasing inclusion of the Pro-vitamin-A enriched cassava flour into wheat flour significantly increases the dietary fibre, mineral content, vitamin A and ascorbic acid of the flour blend samples. 100ACF recorded the highest pro-vitamin A carotenoids and retention followed by 80ACF/20WF. The cyanogenic potential of the composite flour samples were quite below the FAO/WHO recommended safe level of 10ppm. Therefore the developed ACF/WF composite flour samples depending on the degree of supplementation needed could be used by bakers in many baking products, crunches and local diet formulations to improve food nutritional qualities. They could also serve as cheap dietary approach towards prevention, elimination of vitamin A deficiency and its associated diseases and promotion of healthy population. The data generated will provide a basis for public enlightenment programmes to create awareness on the feasibility of production and commercialization of the composite flour samples using the newly released biofortified cassava variant (IBA154810). This will consequently target the promotion and encouragement of small and medium scale industries, reduce the rate of unemployment and improve the economy of the nation.

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ORCID

AmooIsiakaAdekunle: <https://orcid.org/0000-0002-1408-5434>

Ganiyu Oboh: <https://orcid.org/0000-0001-5167-9779>

Stephen AdeniyiAdefegha: <https://orcid.org/0000-0003-1339-403X>

IladeOdunayo Josephine: <https://orcid.org/0009-0003-3153-1568>

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