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Leafy vegetables fortification enhanced the nutritional profile and reduced the glycemic index of yellow cassava pasta

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Food-to-food fortification of yellow cassava flour with leafy vegetable powders (*Amaranthus* and *Telfairia occidentalis*) was employed in this study to develop cassava-vegetable spaghetti-like pasta products (YP, YPA5, YPA10, YPU5, YPU10, YPA5O). The nutritional profile, micronutrient retention, bioaccessibility, starch digestibility and *in vitro* glycemic index were assessed. The incorporation of leafy vegetable powder enhanced the nutritional quality of the yellow cassava pasta (YCP) products. The fortification increased (up to 3-fold) the protein in fortified YCP, increased the fibre (11%), doubled the ash and increased the beta-carotene (about 7-fold), iron (72%) and zinc contents by 10%. The phenolic content of fluted pumpkin leaf-fortified pasta with 10% leaf powder inclusion (YPU10) was 1100 µg GAE g⁻¹, almost four times higher than that of the unfortified YCP. Leaf powders in the cassava pasta also favoured the retention of micronutrients during cooking and slowed down the starch digestibility. The retention during cooking was up to 91% in YPU10 for beta-carotene with no loss in iron, while the bioaccessibility of beta-carotene was impeded, the zinc retention was high and became significantly more bioaccessible with leaf addition and cooking. The estimated glycemic index of YCP was reduced by 19% and 15% in YPU10 and YPA10, respectively. The inclusion of the vegetables also reduced the glycemic index of the fortified YCP. Thus, adding leafy vegetable powder up to 10% into YCP is a promising approach to both valorise yellow provitamin A biofortified cassava and enhance the nutritional value.

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is an important African staple crop. However, the conventional, white-fleshed variety is deficient in protein and several micronutrients, of which vitamin A, iron, and zinc are especially lacking in cassava-based diets.¹ Thus, yellow-fleshed cassava varieties, biofortified with provitamin A carotenoids and low in cyanide, were developed in large-scale breeding programmes.² These varieties provide nutritional benefits over the white-fleshed cultivars.^{1,3} Thus, since 2011, provitamin A biofortified cassava varieties are being promoted and have received appreciable consumer acceptance in several sub-Saharan African (SSA) countries.⁴ Yellow cassava shows excellent potential to alleviate vitamin A deficiency but is still poor in iron and zinc. Moreover, studies revealed post-processing losses of beta-car-

tene up to 70% in yellow cassava products.^{5,6} In SSA, leafy vegetables are abundant, affordable and good sources of essential amino acids, fibre, vitamins and minerals (especially iron and zinc).⁷ Leafy vegetables are commonly prepared by boiling, stewing, frying and blanching, and eaten as a sauce or so-called soup with starchy staples such as cassava. Despite the health benefits, the current consumption of vegetables is insufficient to meet the daily requirements of people living on cassava-based diets.⁷ Raaijmakers *et al.*⁸ and Bakker *et al.*⁹ also reported the low vegetable intake in Nigeria and Rwanda. Thus, fortifying starchy staples with leafy vegetable powder provides an effective and convenient means of ensuring vegetable consumption among the consumers. Food-to-food fortification is thus an emerging approach, mostly implemented in the developing world, to complement other strategies in combating micronutrient deficiencies.^{10,11} To date, this approach has not been used with biofortified yellow cassava. Based on compositional data, a food product combining leafy vegetables and yellow cassava into a convenient and popular food, such as pasta, seems a way to address the still prevailing nutritional deficiencies. Leafy vegetables can be dried and made into leaf powder, simplifying the possibilities for food-to-food fortifica-

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tion of staple foods with leafy vegetables¹² such as fluted pumpkin (*Telfairia occidentalis*) and amaranth (*Amaranthus cruentus*), the two most preferred leafy vegetables in Nigeria.³

Previous studies examined the rich nutritional profile of these leafy vegetables. Lawal *et al.*¹³ reported that *Amaranthus* contain 12.6 g per 100 g protein, 3.3 g per 100 g crude fibre, 6.4 mg per 100 g iron, and 1.7 mg per 100 g zinc on dry weight basis while Akwaowo *et al.*,¹⁴ noted that *Telfairia occidentalis* fresh leaves contain 22.4 g per 100 g protein, 14.2 g per 100 g crude fibre, 3.6 mg per 100 g iron, and 4.2 mg per 100 g zinc. The superior nutritional profile and the blood-glucose-lowering effect of these two vegetables have been reported by various authors^{15,16} thus their hypoglycemic activities could be utilised to produce functional food product.

This food design strategy meets the demand for functional foods with added health benefits, which is increasing worldwide due to growing consumer awareness of the role of these foods in preventing chronic diseases.¹⁷ Inclusion of dried leafy vegetables could also promote the development of low-glycemic-index (below 50) foods resulting in a slower rise in blood glucose and insulin level.¹⁸ In this respect, wheat-based products have been widely investigated in the last 50 years and research is still ongoing about the impact of the quality of gluten network on the glycemic index (GI). The research was boosted by the rising popularity of gluten-free products, not only for celiac people but for all consumers wishing to reduce gluten in their diet. This is an interesting opportunity for cassava-based products particularly gluten-free pasta whose market share is steadily growing.¹⁹

Pasta products are increasingly used as a carrier of functional ingredients in food fortification to enhance nutritional quality, improve health and reduce the risk of diseases.²⁰ As a result of increasing urbanisation and changes in food habits, the pasta market is growing in the low- and middle-income countries of Africa. Several pasta products (*e.g.*, macaroni, spaghetti, noodles) are becoming more popularly consumed in Africa within the last decade due to their delicious taste, extended shelf life, convenience and affordability^{21–23} Nigeria, for instance, has become the 12th largest (<https://instantnoodles.org/en/noodles/market.html>) instant noodle market in the world, with 1.76 billion servings of noodles annually²⁴ while pasta has become a 'street food in several African countries'.^{25,26}

Several authors evaluated the fortification of wheat-based pasta with leaf powder and reported the improvement of the nutritional profile of the functional pasta. Borneo & Aguirre,²⁷ reported that fortification of wheat-based pasta with amaranth leaf powder improved the nutritional composition of the functional pasta while Cárdenas-Hernández *et al.*,²⁸ noted that the inclusion of amaranth flour and leaf powder enhanced the fibre and mineral profile of the fortified pasta. Simonato *et al.*,²⁹ also described how moringa leaf powder enhanced the antioxidant activity, phenolics and mineral content of wheat pasta.

The unique combination between increasing consumption of cassava pasta in countries where this crop is a staple food

and the interest in gluten-free alternatives in Western countries prompted us to study the techno-functional characteristics of YCP fortified with leafy vegetables powders.^{13,21} Yellow cassava, having no gluten network to entrap the starch granules, has a high GI.^{30,31} However, we hypothesized that the insoluble dietary fibre of the leafy vegetables can also create a network to delay starch hydrolysis while the polyphenols in the vegetables can also reduce amylase activity. In principle, the ideal vegetable-fortified pasta product should have a low GI in combination with a high bioaccessibility of the micronutrients from the food matrix during digestion.

Consequently, this study aimed at (1) evaluating the effects of the addition of leaf powder (amaranth and fluted pumpkin leaves) on the retention and bioaccessibility of beta-carotene, iron and zinc in vegetable-fortified YCP, and (2) assessing the impact of the addition of leaf powder on the starch digestibility and *in vitro* GI of the leafy vegetable-fortified YCP.

2. Materials & methods

2.1. Materials

Yellow cassava flour made from low-cyanide sweet cassava variety (TMS 07/0593) was supplied by the International Institute for Tropical Agriculture (IITA) in Ibadan, Nigeria. The leafy vegetables, amaranth (*Amaranthus cruentus*) and fluted pumpkin (*Telfairia occidentalis*) leaves were cultivated at the Unifarm of Wageningen University and Research, The Netherlands. The leaves were harvested in the year 2020, ten weeks after planting, and washed, freeze-dried at -65 °C and milled to ~5 µm particle sizes (6875D Freezer/Mill®, SPEX SamplePrep, UK). All the materials were packaged in amber bottles and stored at -20 °C until they were required for analysis. The chemicals used in the analyses were of analytical grade.

2.2. Methods

2.2.1. Pasta preparation and processing. We prepared pasta samples from yellow cassava flour by incorporating amaranth and fluted pumpkin dry leaf powders at diverse levels as YP (unfortified YCP), YPA5 (YCP with 5% amaranth leaf powder), YPA10 (YCP with 10% amaranth leaf powder), YPU5 (YCP with 5% fluted pumpkin leaf powder and YPU10 (YCP with 10% fluted pumpkin leaf powder with the composition as described in Table 1. The yellow cassava flour and leaf powders were weighed and mixed manually to achieve homogenous mixtures. The dry formulation was then mixed with boiling water in the ratio of 1:1 to form a dough which was manually kneaded and allowed to rest for 20 min. Long pasta strands (spaghetti-like) were produced with a small-scale manual pasta extruder (CuisinU RVS Compact Pasta machine, Roelofsarendsveen, the Netherlands) and laid out on an aluminium foil. The pasta was then dried in an incubator at 60 °C for 5–6 h. The pasta samples were stored in plastic amber bottles at -20 °C until needed for analysis. The pasta samples were weighed and cooked until the white core disappears using the method as described by Lawal *et al.*²¹ After cooking,



Table 1 Composition of YCP

Sample code	Composition
YP	100% yellow cassava flour, 0% leafy vegetables
YPA ₅	95% yellow cassava flour, 5% amaranth vegetable
YPA ₁₀	90% yellow cassava flour, 10% amaranth vegetable
YPU ₅	95% yellow cassava flour, 5% <i>fluted pumpkin leaf</i> vegetable
YPU ₁₀	90% yellow cassava flour, 10% <i>fluted pumpkin leaf</i> vegetable
YPA ₅ O	95% yellow cassava flour, 5% amaranth vegetable with oil

YP (YCP), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta), YPA₅oil (YCP with 5 g amaranth leaf powder per 100 g of pasta with the addition of one teaspoon sunflower oil during cooking).

the pasta was strained, cooled and weighed while 5 ml of sunflower oil was added to the water for cooking sample YPA₅ O (YCP with 5% amaranth leaf powder plus 5 ml oil) to assess the impact of oil addition on the measured parameters.

2.2.2. Proximate composition, elemental analysis, and colour measurements of YCP. The moisture content (MC) of the YCP and leaf powder samples was determined by oven-drying at 105 °C, and ash content was estimated after incineration of the organic fraction in a muffle furnace at 550 °C overnight according to the method 925.09.³² The protein content (PC) was measured using the Dumas combustion method (EA 1112 NC, Thermo Fisher Scientific Inc., Waltman, USA) to estimate the nitrogen content with a protein conversion factor of 6.25. D-Methionine (ACROS Organics) was used as standard.³³ The Soxhlet petroleum ether extraction system extracted fat from 5 g of the sample according to an AOAC method 925.07.³² Total carbohydrate content was calculated by the subtraction method, *i.e.*, the fraction retained after deduction of other proximate compositions on a g per 100 g dry weight basis. The total dietary fibre (TDF) was determined using the Official Method 991.43³² while the total energy value was calculated per 100 grams of the sample using the Atwater conversion values. The sugar content was measured by HPLC based on AOAC 977.20 with minor modifications.³⁴ The Apparent amylose content (AAC) of the pasta was determined following a modified method based on an iodine colourimetry method described by Man *et al.*³⁶ The iron and zinc contents of the pasta and leaf powders were determined using inductively coupled plasma mass spectrometry ICP MS NEN-ISO 17053 according to AOAC 999.10.³⁵ Prior to the analysis, samples were dried at 70 °C overnight and ground to 0.425 mm. Then, 300 mg of the ground sample was digested with concentrated nitric-hydrochloric acid mixture, and hydrogen-peroxide in a microwave digestion system (MarsXpress; CEM Corporation, Matthews, USA). After settling of the undissolved silica particles, the supernatant was analysed on the ICP-AES. mm and measurements were based on mg per kg dry weight.

The colour of the pasta samples was measured using a Hunter Lab flex colourimeter (Elscolab, USA).

The parameters were recorded as L^* (lightness: $L^* = 0$ black and $L^* = 100$ white), a^* (redness-greenness: $-a^* =$ greenness

and $+a^* =$ redness) and b^* (yellowness-blueness: $-b^* =$ blueness and $+b^* =$ yellowness) values. The colourimeter was standardised with a white plate supplied with the equipment, and three readings were done for each sample.

2.2.3. Determination of total phenolics, flavonoids and antioxidant activity. Extraction of phenolics and flavonoids of all the samples was performed as described by Li *et al.*³⁷ with few modifications. 2.0 g of finely ground pasta samples were extracted with 80% acidified (0.1%) methanol by refluxing twice in a shaking water bath (SW23 JULABO GmbH, Germany) at 40 °C for 2 h and centrifuged at 2000g for 10 min in a centrifuge (Thermo Scientific Multifuge X3R Refrigerated Centrifuge, Marshall Scientific, USA). The rotary evaporator (Büchi Rotavapor® R-205, Marshall Scientific, USA) was used to evaporate the remaining water while methanolic extracts were freeze-dried and stored at 4 °C until needed for further analysis. The total phenolic content of the samples was determined by the Folin-Ciocalteu colourimetric method as described in AOAC 2017.13.³² The result was expressed as µg of gallic acid equivalent (GAE) per g of the sample. Total flavonoid was determined as Li *et al.*³⁷ described and results were expressed as mg Rutin equivalent (RE) per g of sample. Antioxidant activity of pasta and leaves powders extracts was assessed in terms of DPPH (2,2 diphenyl-2-picrylhydrazyl) radical scavenging activity using the method of Plank *et al.*,³⁸ with an adaptation of the Quencher method.³⁹ 0.1 mL of methanolic extracts were added to freshly prepared 3.9 mL of 0.2 mM DPPH solution at different concentration (5, 10, 15, 20, 25, 30 µg ml⁻¹) followed by incubation of 30 min in the dark, and absorbance was noted at 515 nm. The result was expressed as the percentage inhibition of the DPPH radical. The percentage inhibition of the DPPH radical was calculated according to the following equation:

$$\begin{aligned} \% \text{ Inhibition of DPPH} = & (\text{Abs control} \\ & - \text{Abs sample}/\text{Abs control}) \times 100 \end{aligned} \quad (1)$$

where: Abs control is the absorbance of the DPPH solution without the extract.

2.2.4. Beta-carotene content evaluation. The determination and quantification of beta-carotene content were carried out using Sadler, Davis & Dezman⁴⁰ method with slight modifications. The extracted samples were filtered and the beta-carotene (Sigma C 4582) standard of different concentrations (0.313, 0.625, 1.25, 2.5, 5, & 10 µg ml⁻¹) were injected into the HPLC column. The beta-carotene content was analysed using an HPLC-system (Water Corporation, Milford, MA, USA) consisting of a guard column, C30 YMC Carotenoid column (4.6 × 150 mm, 3 µm) supplied by YMC Europe (GMBH, Dinslaken, Germany). A 2996 photodiode array detector (PDA) was used for the beta-carotene quantification. Chromatograms were generated at 450 nm and subsequent identification of *cis* and *trans* isomers of beta-carotene was made using the reference substance materials (Appendix 1). The HPLC analysis was carried out using the isocratic gradient at a run of 25 min



while the mobile phase was acetonitrile: methanol: ethyl acetate in 60:30:10 ratio with 0.01% triethylamine (TEA).

2.2.5. Percentage retention of beta-carotene, iron and zinc in yellow cassava pasta. The effect of cooking on the retention of beta-carotene, iron and zinc contents in the YCP samples (cooked and uncooked) were calculated using the percentage true retention (TR) formula developed by Lee *et al.*⁴¹ TR percentage values are used to determine the total proportion of carotenoids lost during processing:

$$\text{True retention}(\%) = \frac{\text{Nutrient content of cooked pasta} \times \text{g of pasta after cooking}}{\frac{\text{Nutrient content of raw pasta} \times \text{g of pasta before cooking}}{\times 100}} \quad (2)$$

2.2.5. Determination of the bioaccessibility of beta-carotene, iron and zinc. The standardised static *in vitro* digestion protocol known as INFOGEST and described by Minekus *et al.*⁴² was used in this study to estimate the bioaccessible fraction of the beta-carotene and minerals. In this protocol, the *in vitro* bioaccessibility was determined after the cooked samples had passed through a simulated gastrointestinal model, which included oral, gastric, and intestinal phases.³² In the oral digestion stage, 3.5 mL of simulated salivary fluid (SSF) electrolyte stock solution was added to 5 g each of sample, followed by 25 μ L of 0.3 M CaCl_2 and 1475 μ L of Milli Q water. The mixture was shaken for 2 min at 37 °C. In the gastric digestion conditions, the above mixture (10 mL) was mixed with 7.5 mL of simulated gastric fluid (SGF) electrolyte stock solution, 1.6 mL porcine pepsin stock solution (25 000 U mL⁻¹), 5 μ L of 0.3 M CaCl_2 , then HCl (1 M) was added to lower the pH to 3.0 and water up to 20 mL. The reaction vessel was placed into a shaking water bath at 37 °C for 2 h. During the intestinal phase, the gastric mixture was mixed with 11 mL of simulated intestinal fluid (SIF) electrolyte stock solution, 5.0 mL of pancreatin solution (800 U mL⁻¹), 2.5 mL of bile salt solution (160 mM) and 40 mL of CaCl_2 (0.3 M). NaOH (1 M) was then added to neutralise the mixture to pH 7.0 and Milli Q water up to 40 mL. Additionally, we also cooked YPA5 samples with 2 mL of sunflower oil to determine the impact of oil on beta-carotene, iron and zinc bioaccessibility. The bioaccessibility was calculated as the percentage of micronutrients present in the supernatant after *in vitro* digestion and centrifugation (IVD), based on the concentration and volumes in the cooked samples (adapted from Oomen *et al.*⁴³).

$$\text{Bioaccessibility}(\%) = \frac{\text{Concentration in IVD supernatant}}{\text{Concentration in the cooked sample}} \times 100 \quad (3)$$

2.2.6. Determination of *in vitro* starch digestibility. The *in vitro* starch digestibility of the YCP products was measured using the method developed by Englyst *et al.*⁴⁴ Digestion of the cooked pasta samples was achieved by incubating with the mixture of purified pancreatic alpha-amylase and amyloglucosidase at 37 °C for 4 h while stirring continuously. The solu-

tion's aliquots (1.0 mL) were removed while stirring at 20 min to measure the Rapidly Digestible Starch (RDS) and at 120 min to measure the Slowly Digestible Starch (SDS). Next, the aliquots were transferred to 50 mM acetic acid solution to stop the reaction. Finally, at 240 min, 4.0 mL of aliquot was removed, added to 4.0 mL of ethanol, and centrifuged. The pellets were washed with aqueous ethanol to remove free glucose and suspended in NaOH to dissolve the Resistant Starch (RS) and measure the Total Digestible Starch (TDS) and RS. D-Glucose was measured separately with glucose oxidase/peroxidase (GOPOD) reagent. The total starch was determined separately using the Total starch assay procedure (Megazyme International, Ireland) as described by Englyst *et al.*⁴⁵ while the SDS and RS were determined with the Digestible & Resistant Starch Assay kit (K-DSTRS, Megazyme International, Ireland). The rapidly available glucose (RAG) values were reported to help predict the glycemic responses of food.⁴⁶ The G20, G120, and TS values were used to calculate RDS, SDS, and RS amounts using the following eqn (4)–(7):

$$\text{Rapidly digestible starch (RDS)} = (G_{20} \times F \times 0.9 \times 100)/W \quad (4)$$

$$\text{Slowly digestible starch (SDS)} = ((G_{120} - G_{20}) \times F \times 0.9 \times 100)/W \quad (5)$$

$$\text{Total starch (TS)} = (G_{TS} \times F \times 0.9 \times 100)/W \quad (6)$$

$$\text{Resistant starch (RS)} = \text{TS} - (\text{RDS} + \text{SDS}) \quad (7)$$

where G_{TS} = absorbance value of total starch, F = 100/GOPOD absorbance, W = sample weight (mg).

2.2.6.1. Estimation of the glycemic index of cassava pasta samples. A first-order non-linear equation model described by Goñi *et al.*⁴⁷ was applied to describe the kinetics of starch hydrolysis and the estimate glycemic index (GI) of the cooked pasta samples where $C = C_{\infty} (1 - e^{-kt})$ where C represented the percentage of starch hydrolysed at time t (min), C_{∞} is the maximum hydrolysis extent, and k is the kinetic constant.⁴⁸ The parameters, C and k , were estimated for each cassava pasta sample based on the *in vitro* starch digestion data. The hydrolysis index (HI) was then calculated by dividing the Area Under Curve (AUC) of each starch hydrolysis by the AUC of the reference food (white bread). The HI is expressed as a percentage representing the rate of starch digestion. The estimated GI indicated the digestibility of the pasta starch with the digestibility of starch in the reference material white bread. The estimated glycemic index of YCP was calculated from eqn (8):

$$\text{Estimated GI} = 39.71 + 0.54\text{HI}. \quad (8)$$

2.2.7. Statistical analysis. The experiments were performed in triplicates. First, a one-way analysis of variance (ANOVA) was used to analyse the data, followed by Duncan's multiple range test for mean comparisons using SPSS (version 25.0). Results were expressed as the mean \pm standard deviation, and a *p*-value of <0.05 was considered statistically significant.



3. Results and discussion

3.1. Proximate composition, elemental analysis, colour measurements and apparent amylose content of YCP

3.1.1. Proximate composition of dry leaf powder and yellow

cassava pasta. Table 2 shows the chemical compositions of the dry leaf powder and the YCP (fortified and unfortified). The moisture content of all the YCP samples was <12.0 g per 100 g, in line with the regulatory standards for dried pasta (12–13%).⁴⁹ The impact of leaf powder inclusion on the moisture content of YCP was however minimal ($<1.0\%$). Low moisture content is needed in pasta products because it limits microbial growth, off flavour and rancidity, as pasta is commonly supplied in the dry state to ensure storage stability and transportation.⁵⁰ Proximal composition of the amaranth and fluted pumpkin leaf powders in this study confirmed the higher content of protein (31.49 ± 1.44 & 34.70 ± 0.02 g per 100 g), ash (15.4 & 15.8 g per 100 g), and total dietary fibre (11.2 & 12.15 g per 100 g) than those reported in previous literature⁵¹, likely due to differences in analytical methods, the cultivar and planting conditions. Schönfeldt *et al.*⁵² & Ogunttoyinbo *et al.*⁵³ found 2.4–3.5 g per 100 g protein, 1.6–2.4 g per 100 g ash, 6.7 g per 100 g fibre in fresh amaranth leaves while Aworh *et al.*⁵⁴ reported 6.1% protein, 1.7% ash and fibre contents in fluted pumpkin fresh leaves. Islam *et al.*⁵⁵ however reported comparable protein content in Moringa dry powder (29.4 g per 100 g). The high protein content of leafy vegetables powders enhances their value as important ingredients in food-to-food fortification.⁵⁶ The addition of amaranth and fluted pumpkin leaf powders in this study thus enhanced the protein content of the developed pasta, and the fortified YCP had about double the protein content (up to 2.95 g per 100 g) of unfortified YCP (0.99 g per 100 g) as shown in Table 2. The fluted pumpkin leaf-fortified cassava pasta also had a higher protein content (2.9–3.0 g per 100 g) than the amaranth-fortified YCP (2.5 g per 100 g–2.7 g per 100 g) due to the higher protein of the fluted pumpkin leaf powder. The protein level is thus affected by the type of leaf powder used in fortification. The higher level of protein in the YCP is particularly desirable but lower than the results obtained by Borneo & Aguirre,²⁷ in wheat-based amaranth-fortified pasta (14.2%). One of the weak nutritional features of cassava food products is the low level of protein, which is lower than in wheat-based pasta.

The food-in-food strategy however moderately improved the dietary fibre content of the YCP samples from 9.0 to 10.0 g per 100 g, mainly due to the yellow cassava flour's high fibre and resistant starch content rather than the high fibre in the leafy vegetables. The dietary fibre content of fortified YCP samples were thus slightly improved with leaf powder fortification. Sato *et al.*⁵⁷ however reported higher protein levels when dried leaves of *Pereskia aculeata* Miller were utilised to fortify wheat-based pasta (1.9 to 10.2%). The intake of dietary fibre rich products is desirable to meet nutritional recommendations as it helps in the proper control and management of diabetes and obesity. The structure provided by the dietary fibre network

Table 2 Chemical and mineral composition of leaf powders and YCP samples

The data expressed as mean values \pm standard deviation was of three independent experiments on a dry weight basis ($n = 3$). Different superscript letters on a row indicate significant differences among samples ($p \leq 0.05$), nd - not determined, ND* - not detected, YP (YCP), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta), YPA₅oil (YCP with 5 g amaranth leaf powder per 100 g of pasta with oil added during cooking), ALP (amaranth dry leaf powder), ULP (fluted pumpkin leaf powder), (moisture content, protein content, fat content, ash content, total dietary fiber) in g per 100 g on a dry weight basis, CH (C₆H₁₀O₅) carbohydrate content calculated difference, E (Ergocalciferol) content expressed in μ g per 100 g, mineral composition (iron content and zinc content) in mg kg⁻¹ on a dry weight basis, sucrose content (fructose, glucose, sucrose) in mg σ^{-1} apparent amylase content expressed in percent.

can control the kinetic of glucose release in the blood.⁵⁸ The ash content of fortified pasta ranged from 2.3 g per 100 g–5.1 g per 100 g (dry weight), which was significantly different ($P > 0.05$) from the unfortified yellow pasta YP (1.6 g per 100 g), a consequence of the high ash content of cassava. This ash content was higher than those obtained by Sato *et al.*,⁵⁷ when 10% *Pereskia aculeata* Miller leaf powder was incorporated into wheat pasta (2.2 g per 100 g). Generally, ash, protein and dietary fibre contents were lower in the control unfortified YCP than the fortified pasta samples while a higher level of fortification with leaf powder also enhanced the nutritional value of the pasta.

3.1.2. Mineral contents of leaf powders and yellow cassava pasta. Leaf powders are known as very good sources of minerals⁵⁴ hence the incorporation into commonly consumed foods is an innovative approach to boost vegetable intake.⁵⁹ The mineral content of leafy vegetables differs widely, as our results showed that fluted pumpkin leaf powder contained higher iron content (110.5 vs. 97.0 mg per kg dw) but lower zinc (6.7 vs. 49.0 mg per kg dw) than amaranth leaf powder. In comparison, the zinc content of amaranth was six-fold higher than found in fluted pumpkin leaf powder. This agrees with previous reports of amaranth vegetables being notably rich in zinc⁶⁰ (6.0 mg kg⁻¹) and fluted pumpkin leaf's superior iron content (96.0 mg kg⁻¹) among other leafy vegetables.⁵⁴ Fortification of YCP with dry leaf powders amaranth and fluted pumpkin thus enhanced the iron (from 25.0 up to 43.0 mg per kg dw) and zinc (from 9.1 up to 10.0 mg per kg dw) contents of the cassava pasta products (Table 2). A higher increase was however found for iron (up to 72% in YPU10) than zinc (10% in YPA10). The mineral content of YCP was enhanced by the vegetable fortification.

3.1.3. Apparent amylose content of yellow cassava pasta. The apparent amylose content of YCP was significantly different among the samples ranging from 21.9 to 30.9, with yellow pasta fortified with 10% fluted pumpkin leaves (YPU10) having the highest value (Table 2). The starch characteristics such as the amylose/amylopectin ratio, the plant species and the presence of other food components with their interactions during processing, determine the digestibility of the starch.⁶¹ Thus, starch types with a high amount of amylose are used as a source of resistant starch (RS) while high amylose in pasta samples is desirable. In addition, previous literature reported that starchy foods rich in amylose are associated with a drop in blood glucose levels and more gradual emptying of the human gastrointestinal tract *versus* those with low levels of amylose.⁶² The amylose content of the YCP thus depend on the starch characteristics. Fortification with leaf powder also affected the amylose content as an increase was observed with higher level of substitution with the leaf powder.

3.1.4. Colour profile of yellow cassava pasta. The pasta's colour profile as presented in Table 3 showed that the addition of leaf powder significantly influenced the colour attributes of the fortified pasta. The quality of pasta could be estimated from its colour as it is a crucial factor impacting consumer preference for pasta and is known to change during processing.⁶³

Table 3 Colour of the YCP (fortified and unfortified)

	<i>L</i> *	<i>a</i> *	<i>b</i> *
YP	70.0 ± 0.7 ^d	1.2 ^e ± 0.1	15.1 ^b ± 0.3
YPA ₅	79.7 ^e ± 0.4	-7.3 ^b ± 0.1	11.2 ^a ± 0.1
YPA ₁₀	66.2 ^c ± 0.0	-7.9 ^a ± 0.2	16.6 ^c ± 0.2
YPU ₅	58.5 ^b ± 0.1	-6.4 ^d ± 0.1	26.2 ^d ± 0.1
YPU ₁₀	52.0 ^a ± 0.0	-6.7 ^c ± 0.0	27.1 ^c ± 0.1

Mean ± SD, $n = 3$, columns with different superscripts are significantly different ($p < 0.05$) *L** scale: 0–50 (dark); 51–100 (light); *a** scale: +ve value (red); -ve value (green); *b** scale: +ve value (yellow); -ve value (blue). YP (YCP), YPA₅ (YCP with 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta), YPA_{5oil} (YCP with 5 g amaranth leaf powder per 100 g of pasta with the addition of one teaspoon sunflower oil during cooking).

Traditionally, semolina-based pasta exhibited light yellow colouration derived from beta-carotene.⁶⁴ Thus, the YCP, biofortified with beta-carotene and bright yellow (Fig. 1), appeared most similar in colour to the wheat-based pasta. The *L** value, which is the most critical colour parameter related directly to the preferred brightness, decreased significantly among the pasta samples from 70.0 in YP to 52.0 in YPU10 ($p < 0.05$) due to the incorporation of the leaf powder. As expected, a pronounced greenness was observed in all the fortified pasta due to chlorophyll in the leaf powder. Colour losses were also observed after cooking the pasta due to the slight diffusion of the pigments into the cooking water, similar to the findings of Simonato *et al.*²⁹ with moringa leaf powder fortified wheat pasta. Furthermore, the *b** value of the pasta samples increased with higher level fortification while those fortified with fluted pumpkin had significantly higher *b** values than those fortified with amaranth leaf powder. More precise analyses of YCP colours can however be achieved using a colour analyser.

3.2. Total phenolics, flavonoids and antioxidant activity of YCP and leaf powders

The phenolic contents of the YCP samples varied according to the presented food matrix and ranged from $226.6 \pm 2.22 \mu\text{g GAE g}^{-1}$ in YCP to $1098.3 \pm 5.26 \mu\text{g GAE g}^{-1}$ in YPU10 (Table 4), showing that the leaf powder addition significantly ($p \leq 0.05$) increased the total phenolics content of fortified YCP. Thus, the highest phenolic content was found in the fluted pumpkin leaf-fortified pasta YPU10 and was almost four times higher than found in the unfortified YCP. Phenolic compounds have excellent antioxidant properties due to their ability to bind metal ions, reduce peroxides, and promote the potency of anti-oxidative enzymes.⁶⁵ They may also inhibit digestive enzymes (α -amylase and amyloglucosidase) through chemical interactions that lead to precipitation of the enzymes, thus limiting their activity on the digestion of carbohydrate foods. Moreover, starch-phenolics complexes have been reported to significantly slow down starch digestion.⁶⁶ Fluted pumpkin and amaranth leaves are particularly rich in



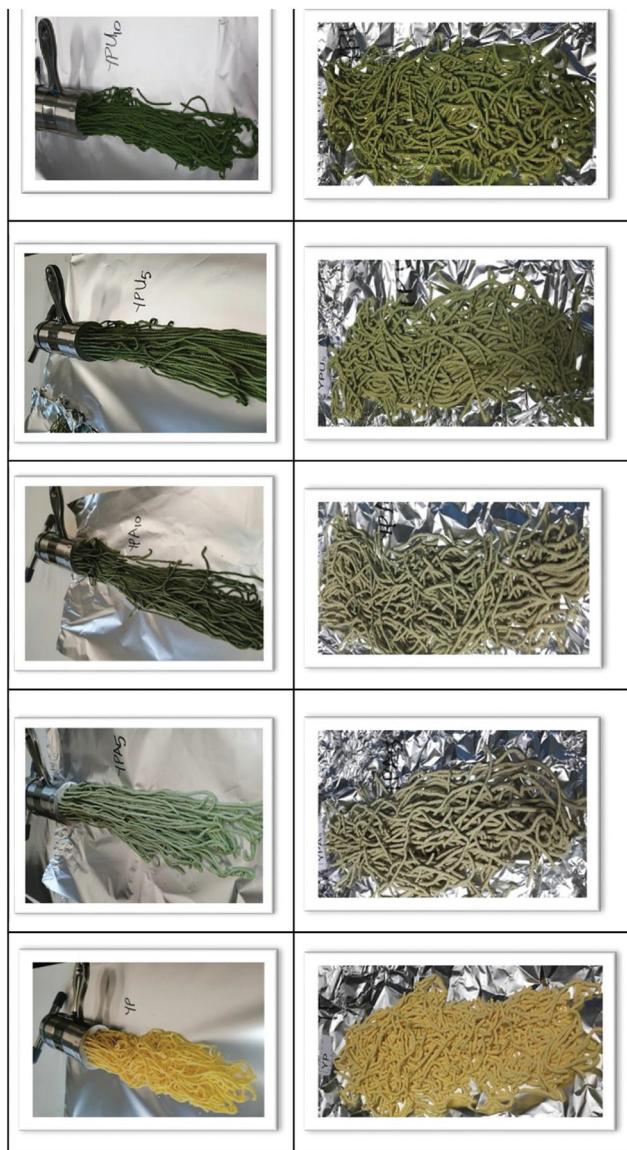


Fig. 1 Yellow cassava pasta samples (fortified and unfortified). Left (samples after extrusion), right (samples after cooking), bottom-up: YP (YCP), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta).

flavonoids, the largest and most abundant group of secondary metabolites with marked antioxidant properties in leafy vegetables.⁶⁷ In this study, fluted pumpkin leaves were found to contain higher total phenolics (931.1 GAE μg per g DW) than the amaranth leaves (862 GAE per μg DW) but fewer flavonoids (101.5 μg RE per g DW) than the amaranth leaves (111.1 μg RE per g DW). This is higher than values reported by other authors for amaranth⁶⁸ (total phenolics 14.8 GAE per μgg FW, total flavonoids 62.5 RE per μgg DW) while Okonwu *et al.*,⁶⁹ reported 7.7–13.4 g per 100 g flavonoids in fluted pumpkin leaves. The highest flavonoid content was found in the fluted pumpkin fortified pasta YPU10 (80% higher than in the unfor-

tified sample). In comparison, the highest antioxidant activity as measured by the DPPH free radical scavenging activity among the pasta samples was observed for the amaranth-fortified pasta, YPA10 (0.98 mmol kg^{-1}) which is 122% higher than in unfortified YCP. The DPPH profile of the YCP reported in this study suggests significant antioxidant activities thus a comparison method such as ABTS (2,20-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid)) with Butylhydroxytoluene (BHT) used as standard antioxidant in the experiments could be used in future research to validate the results and the IC₅₀ values should also be determined.

3.3. Beta-carotene content of leaf powders and YCP (fortified and unfortified)

Beta-carotene accounts for more than 90% of the total carotenoids in vegetables,⁷⁰ thus the fortification of YCP with beta-carotene rich leafy vegetables (amaranth-9.4 $\mu\text{g g}^{-1}$ and fluted pumpkin leaf -13.6 $\mu\text{g g}^{-1}$) enhanced the beta-carotene contents of the YCP (Table 4). The beta-carotene content of YCP was thus improved with increasing leaf powder fortification, as beta-carotene was six-fold higher (2.5–4.3 $\mu\text{g g}^{-1}$) in the fortified than in the unfortified YCP. However, beta-carotene found in the YCP were mainly in *trans*-form while the *cis*-isomers were mostly below detection limits due to the analytical methods employed. Processing such as boiling, steaming, stir-frying, blanching or par-boiling has been reported to result in the formation of *cis*-isomers which possess different biological properties such as decreased provitamin A activity and altered bioavailability and antioxidant capacity.⁴¹ A limitation of this analysis is thus the poor detection of the *cis*-isomers and the interference/non-separation of the chlorophyll content of the leafy vegetables (Appendix 1) as well as the lack of evaluation of their influence on the shelf life of YCP. Future studies into the effect of chlorophyll on the storability of the YCP and a more in-depth study of the *cis*-isomerization or degradation is thus recommended.⁷¹

3.4. Retention and bioaccessibility of beta-carotene, iron and zinc in cooked yellow cassava pasta

The effect of cooking on the retention of beta-carotene, iron and zinc in the cooked YCP are shown in Table 5. As previously reported, processing (boiling) led to losses in beta-carotene content of yellow cassava food products.^{6,72} The fortified YCP however showed higher retention of beta-carotene (60–91.6%) than the unfortified YCP (11.1%), an indication of the carotenoid stability conferred on the product by the addition of leaf powder. The values are higher than beta-carotene retention values reported by Taleon *et al.*⁷³ for other processed yellow cassava products such as *fufu*-fermented porridge (21.6–35.7%), *chickwangue*-stiff dough (1.5–5.6%). As reported by Lawal *et al.*⁶ *gari* and *pupuru* (traditional products of cassava in Nigeria), similarly had low beta-carotene retention (24.4–35.2% and 34.7–39.9%, respectively), while Eyinla *et al.*⁵ reported an even lower β -carotene retention in yellow cassava chips (13.7%), flour (11.7%) and dough (5.5%) while Vimla *et al.*,⁷² showed that yellow-fleshed cassava retained 51.3–81% and 44.1–83.9%

Table 4 Total phenolics, flavonoids, antioxidant and beta-carotene contents of leaf powders and cooked YCP

Parameters	DPPH (mmol kg ⁻¹) DW	Total phenolics (GAE µg g ⁻¹) DW	Total flavonoids (RE µg g ⁻¹) DW	Beta-carotene (µg g ⁻¹) DW
YP	0.44 ± 0.04 ^b	226.6 ± 2.22 ^c	34.1 ± 0.15 ^c	0.48 ± 0.01 ^{bc}
YPA5	0.88 ± 0.02 ^a	445.17 ± 3.23 ^b	58.0 ± 0.09 ^{ab}	2.11 ± 0.09 ^b
YPA10	0.98 ± 0.12 ^a	449.97 ± 2.18 ^b	57.8 ± 0.07 ^b	3.81 ± 0.08 ^{ab}
YPU5	0.41 ± 0.04 ^{bc}	893.05 ± 3.77 ^{ab}	57.7 ± 1.05 ^b	2.91 ± 0.01 ^{ab}
YPU10	0.73 ± 0.16 ^b	1098.32 ± 5.26 ^a	61.6 ± 0.05 ^a	7.82 ± 0.23 ^a
YPA5 O	0.87 ± 0.06 ^{ab}	432.08 ± 1.21 ^b	55.3 ± 0.32 ^{bc}	1.50 ± 0.14 ^b
*ALP	1.75 ± 0.03	862.02 ± 4.15	111.5 ± 0.10	9.40 ± 0.03
*FLP	2.83 ± 0.01	931.10 ± 3.12	101.5 ± 0.09	13.60 ± 0.04

Values are presented as means or range; different superscript letters (each column) represent significant differences ($p < 0.05$). YP (yellow cassava pasta), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta), YPA_{5oil} (YCP with 5 g of amaranth leaf powder per 100 g of pasta with oil added during cooking) *separately analysed ALP (amaranth leaf powder), FLP (fluted pumpkin leaf powder).

of beta-carotene after boiling and stir-frying. Iron retention in the cooked vegetable-fortified YCP was from 62.5 to 100% in line with Pereira *et al.*⁷⁴ who also reported high retention of iron (>94%) in solar dried amaranth leaves. The zinc content of the fortified YPU10 was also substantially improved on cooking, as similarly reported by other authors, probably due to the leaching of the cooking pots into the cooked samples.⁵⁹

The bioaccessibility of beta carotene was however impeded by the addition of leaf powder mostly due to the presence of the bioactive compounds. Similar low bioaccessibility of beta-carotene had been reported (<0.15%) in leafy vegetables and

(0.6% to 3.0%) in orange-fleshed sweet potatoes.^{75,76} In contrast, higher bioaccessibility of beta-carotene ranging from 8% to 40% was reported by Berni *et al.*⁷⁷ for orange-fleshed sweet potato. Iron bioaccessibility was also found to be low in this study (20.9–36.4%) while zinc was significantly more bioaccessible. Icard-Vernière *et al.*⁶⁰ in their study on bioaccessibility of iron and zinc in leafy vegetables, reported up to 17% bioaccessibility of iron in amaranth vegetable sauces while the study by Gautam *et al.*⁷⁸ showed up to 193% bioaccessibility for zinc in cereal food products. Kruger *et al.*,¹⁰ also reported increased bioaccessible iron by 519% and zinc by 295% with the addition of *Vigna unguiculata* leaves to whole grain maize porridge. The addition of oil to YCP did not show any significant effect on bioaccessibility of the micronutrients, as the % bioaccessibility values of beta carotene, iron and zinc in YPA5 and YPA5 O were similar. Since the beneficial effects of bioactive compounds of food depend not only on their content and the amount consumed but also on their bioavailability/bioaccessibility, the nutritional content of food should be ultimately bioavailable/bioaccessible.

Table 5 True retention and bioaccessibility of beta carotene, iron and zinc of YCP

	Uncooked samples	% True retention after cooking	% Bioaccessibility after <i>in vitro</i> digestion
Beta-carotene (µg g⁻¹)			
YP	0.48 ± 0.01	11.1	62.5
YPA5	2.11 ± 0.09	66.6	3.4
YPA10	3.81 ± 0.08	87.8	3.9
YPU5	2.91 ± 0.01	70.1	14.8
YPU10	7.82 ± 0.23	91.6	17.5
YPA5 O	1.50 ± 0.14	60.0	2.8
Iron (mg kg⁻¹)			
YP	25.00	80.00	33.0
YPA5	32.00	100.00	21.6
YPA10	35.00	94.30	36.4
YPU5	34.00	81.80	26.7
YPU10	43.00	81.40	20.9
YPA5 O	33.00	62.50	32.0
Zinc (mg kg⁻¹)			
YP	9.10	53.8	326.5
YPA5	9.80	78.6	194.8
YPA10	10.00	93.0	129.0
YPU5	8.90	71.9	218.8
YPU10	7.30	113.7	168.7
YPA5 O	8.50	58.2	193.0

YP (YCP), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta), YPA_{5oil} (YCP with 5 g of amaranth leaf powder per 100 g of pasta with a teaspoon of sunflower oil added).

3.5. Starch digestibility and *in vitro* glycemic index of YCP

The rapidly available glucose content (RAG) varied significantly among the pasta samples from 21.9–27.6 with the highest in unfortified YCP (Table 6). Conversely, the slowly digestible starch (SDS) was found to be the lowest in the unfortified yellow pasta. The RS for the fortified cassava pasta samples was in the range of 1.78–2.45% (Table 6), higher than Eyinla *et al.*³⁰ reported for traditional unfortified cassava products. RAG and RS content of starchy foods are significant determinants of their glycemic response.⁴⁴ Furthermore, several studies had reported the beneficial impact of RS in starch digestion and its consequent lower glycemic responses. The fractions of RDS varied among the YCP samples (40.9–47.11), with YCP fortified with the highest amount of fluted pumpkin (YPU10), having the lowest value. In this case, leaf addition is significant as the phenolic compounds in the leafy vegetables inhibit starch hydrolysis, slowing down starch digestion, thus lowering the glycemic response. The inclusion of fluted pumpkin leaves was also observed to have a higher



Table 6 Estimated glycemic index and *in vitro* starch digestibility of YCP (% dw)

	RAG	RDS	SDS	RS	Estimated GI
YP	27.60 ± 1.16 ^f	42.23 ± 0.17 ^f	40.90 ± 0.15 ^c	1.12 ± 0.03 ^d	71.72 ± 1.12 ^d
YPA ₅	25.48 ± 0.35 ^e	38.98 ± 1.02 ^e	42.78 ± 1.08 ^b	1.78 ± 0.01 ^c	61.39 ± 0.07 ^c
YPA ₁₀	22.80 ± 0.25 ^{ab}	34.81 ± 0.15 ^b	43.99 ± 0.91 ^{ab}	2.05 ± 0.08 ^c	61.11 ± 0.14 ^b
YPU ₅	24.11 ± 0.23 ^c	36.88 ± 0.18 ^d	45.06 ± 0.75 ^a	2.31 ± 0.11 ^b	59.68 ± 0.33 ^{ab}
YPU ₁₀	21.88 ± 1.30 ^d	33.47 ± 0.03 ^a	47.11 ± 0.61 ^a	2.45 ± 0.07 ^a	58.14 ± 0.15 ^a
YPA _{5oil}	23.38 ± 0.58 ^d	35.77 ± 0.19 ^c	43.01 ± 1.10 ^b	2.23 ± 0.05 ^c	60.19 ± 0.03 ^{cd}

Mean ± SD, *n* = 3, columns with different superscripts are significantly different (*p* < 0.05). RAG (rapidly available glucose), RDS (rapidly digestible starch), SDS (slowly digestible starch), RS (resistant starch). YP (YCP), YPA₅ (YCP 5 g of amaranth leaf powder per 100 g of pasta), YPA₁₀ (YCP with 10 g of amaranth leaf powder per 100 g of pasta), YPU₅ (YCP with 5 g of fluted pumpkin leaf powder per 100 g of pasta), YPU₁₀ (YCP with 10 g of fluted pumpkin leaf powder per 100 g of pasta).

impact than the amaranth leaves on the SDS in the pasta samples (107% higher). Foster-Powell *et al.*⁷⁹ and Arvidsson-Lenner *et al.*⁸⁰ estimated the glycemic index of pasta products in the range between 40 and 78 depending on the processing method and plant material used. In addition, the glycemic index (GI) of a food is influenced by the relative presence of rapidly digested starch and slowly digested starch.⁴⁵ Foods with high SDS (42.8–47.1%), such as the vegetable fortified cassava pasta, are ideal for diabetic patients. Its consumption could help manage diabetes due to its lower RDS and lower glycemic index (58.1–61.4) compared to unfortified YCP (71.7) or bread (100).

4. Conclusions

The fortification of YCP with amaranth and fluted pumpkin leaf powders enhanced the pasta's nutritional profile and can be utilised to combat micronutrient deficiencies (vitamin A, iron and zinc). The inclusion of amaranth and fluted pumpkin leaf powders also boosted the iron, zinc, phenolics and flavonoids contents of the formulated pasta. Furthermore, the addition of amaranth and fluted pumpkin leaf powders delayed the kinetic of glucose digestion as shown by the value of RDS, SDS, RS, TS and RAG. Consequently, it reduced the estimated glycemic index of YCP thus proving useful as a low glycemic index product. However, the low beta-carotene bioaccessibility of YCP require further investigation to ascertain degree of isomerization due to cooking. Also, the impact of chlorophyll on the shelf life of the fortified YCP products should be ascertained. The results obtained from this study showed that incorporating leaf powder in YCP is a feasible way to obtain pasta products with unique micronutrient profiles and improved glycemic response. The YCP as a functional food product is nutritious, convenient and affordable and can help in tackling nutritional deficiencies, especially among low-income consumers in cassava consuming countries.

Compliance with ethical standards

The authors complied with all the ethical standards stipulated.

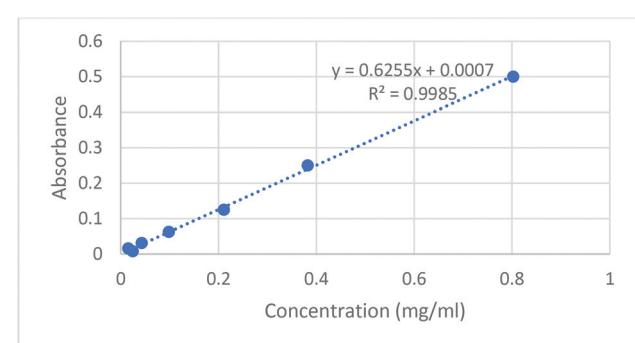
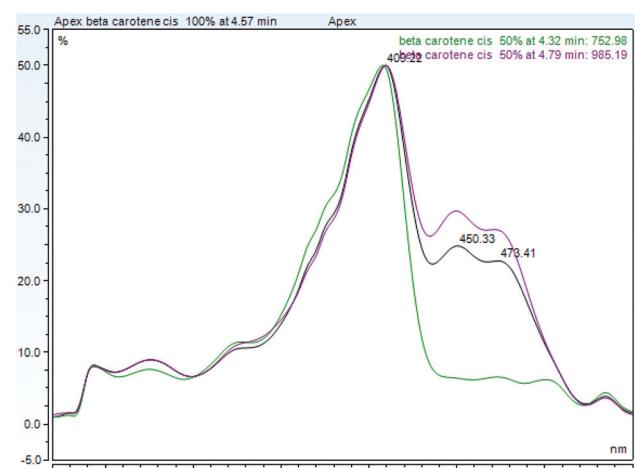
Author contributions

All authors provided feedback on the manuscript and approved the submitted version.

Conflicts of interest

The authors declare no conflict of interest.

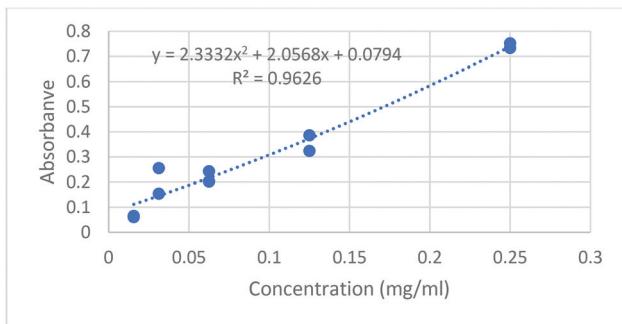
Appendix 1



Phenolics calibration curve.

Phenolics calibration curve.





Flavonoids calibration curve

Flavonoids calibration curve.

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