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# Antioxidant, starch-digesting enzymes inhibitory, and pasting properties of elite yellow-fleshed cassava genotypes

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Biofortification of cassava roots has improved its health benefits by raising the quantity and bioavailability of bioactive compounds, particularly carotenoids. This study analyzed the bioactive constituents (carotenoids, tannins, total phenolics, and flavonoids), antioxidant, starch-digesting enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) inhibitory and pasting properties of flours of 18 elite yellow-fleshed cassava genotypes grown at the International Institute of Tropical Agriculture, Ibadan, using standard laboratory methods. Generally, the concentrations of the bioactive constituents (9-*cis*- $\beta$ -carotene, 13-*cis*- $\beta$ -carotene, all *trans*- $\beta$ -carotene, total  $\beta$ -carotene, total carotenoids, tannins, total phenolics, and total flavonoids) of the different cassava genotypes varied. The antioxidant activity varied significantly among the different cassava genotypes, with IITA-TMS-IBA183001 having the highest reducing power (2.59 GAE mg/g) and most potent DPPH<sup>•</sup> scavenging ability (SC<sub>50</sub>: 14.56 mg/mL). However, the starch-hydrolysing enzymes (alpha-amylase and alpha-glucosidase) inhibitory and the pasting properties of the different genotypes were generally comparable. Total carotenoids content significantly correlated with the DPPH<sup>•</sup> SC<sub>50</sub> ( $r = -0.495$ ), while total phenolics content correlated with ABTS<sup>•+</sup> ( $r = 0.839$ ) and DPPH<sup>•</sup> SC<sub>50</sub> ( $r = -0.870$ ). Also, tannins content significantly correlated with ABTS<sup>•+</sup> ( $r = 0.553$ ), while total flavonoids content was significantly correlated with  $\alpha$ -amylase IC<sub>50</sub> ( $r = -0.532$ ). These findings suggest the potential of flours of the 18 elite yellow-fleshed cassava genotypes to serve as important dietary sources of antioxidants and starch-hydrolysing enzyme inhibitors, which may be beneficial in oxidative stress and postprandial hyperglycaemic conditions.

## KEYWORDS

antioxidant activity, bioactive composition, pasting properties, starch-digesting enzymes, yellow-fleshed cassava

## Introduction

Cassava (*Manihot esculenta* Crantz) roots are the primary calorie sources for ~700 million people living in the world's tropical and sub-tropical regions (Ferraro et al., 2016). Cassava has some agronomic advantages over some other staple crops. It withstands drought stress and soil infertility and thrives in suboptimal conditions. After maturation, it can be

stored underground for several months, without losing its nutrient quality (Ukwuru and Egbonu, 2013). Hence, it is regarded as a viable staple crop for achieving the United Nations Sustainable Development Goal (SDG) of food and nutritional security (Kanter et al., 2016). Traditionally, cassava roots are rich in starch, but lack fat, protein, and micronutrients (Aragón et al., 2018). However, biofortification of cassava roots has improved its micronutrients and bioactive compounds levels, especially provitamin A carotenoids (pVAC). Interestingly, the pVAC level in yellow-fleshed cassava roots has been increased considerably to about 25 µg/g FW, a concentration that surpasses the initial breeding target of 15 µg/g FW (Ceballos et al., 2017).

Presently, pVAC cassava varieties serve as a source of energy. They are also important dietary sources for the alleviation of vitamin A deficiency, especially in developing nations (Aidoo et al., 2022). Furthermore, the carotenoids in yellow-fleshed cassava roots are well-known to possess antioxidant activities. They also protect against some chronic non-communicable diseases including cancer (Agarwal and Rao, 2000) and cardiovascular diseases (Gammone et al., 2017). According to Seifried et al. (2007), the antioxidant activity of carotenoids stands out as the main mechanism underlying their health benefits. Also, Sugiura et al. (2015) reported that consuming carotenoids-rich diets could protect against developing type 2 diabetes (T2D). Aside from the carotenoids in yellow-fleshed cassava, cassava flour also contains phenolic compounds (Ironi et al., 2019; Adefegha et al., 2021), flavonoid glycosides, alkaloids (Osipitan et al., 2015; Pinto-Zevallos et al., 2016), and fiber (Nwose et al., 2017); all of which are prominent for their health benefits. Most of the existing reports on these bioactive constituents, aside carotenoids, were on white-fleshed cassava root and its traditional products, leaving the bioactive components and bioactivities of yellow-fleshed cassava genotypes under-reported. However, this trend is changing as recent research has focused on the bioactive constituents and bioactivity of different traditional products, from biofortified (yellow-fleshed) cassava. For example, our recent study demonstrated the bioactive constituents, antioxidant, and antihyperglycaemic activities of different traditional products (*fufu*, *gari*, and *lafun*) from pVAC biofortified cassava (Kareem et al., 2022). Similarly, Ogbonna et al. (2018) reported the blood glucose response in humans due to the consumption of *gari* produced from different varieties of pVAC biofortified cassava. In a related study, Oluba et al. (2017) investigated the glycaemic index (GI) of *gari eba* produced from pVAC cassava in healthy adult humans. These authors (Oluba et al., 2017; Ogbonna et al., 2018) concluded that the *gari eba* made from pVAC biofortified cassava had a significantly lower GI than that of *gari eba* produced from white-fleshed (non-provitamin A biofortified) cassava roots.

With this available evidence, it is pertinent to appreciate the possible mechanism underpinning the lower GI of pVAC biofortified cassava compared to non-pVAC biofortified cassava. In this context, inhibition of enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) that digest dietary starch by the bioactive constituents in foods is a crucial mechanism to the diminution of postprandial blood glucose response has been attributed (Butterworth et al., 2022; Ironi et al., 2022a). Alpha-amylase and alpha-glucosidase are responsible for catalyzing dietary carbohydrates digestion into simple sugars, and for this reason, their activities affect postprandial

blood glucose levels (Ironi et al., 2019). Furthermore, cassava roots are processed into different products, such as high-quality cassava flour and cassava starch, that have applications in the food and non-food industries. The end-use that these products are suitable for is partly determined by their pasting properties. Pasting properties, including peak, breakdown, trough, final, set back viscosities, peak time, and pasting temperature, represent the behavior of starch when subjected under shear forces to a definite cycle of heating and cooling (Ironi et al., 2019). Hence, to provide insight into the possible mechanism behind the lower GI of pVAC biofortified cassava and its potential industrial uses, this study aimed to determine the bioactive compounds' levels, antioxidant, starch-hydrolysing enzymes inhibitory, and pasting properties of flours of 18 elite yellow-fleshed cassava genotypes.

## Materials and methods

### Samples collection and preparation

Freshly harvested root samples of 18 elite yellow-fleshed cassava genotypes (IITA-TMS-IBA070337, 070593, 182924, 182926, 182959, 182961, 182962, 182963, 182984, 182986, 182993, 183001, 183007, 183008, 183023, 183028, 183037, and 183044) were collected from the research farm of the International Institute of Tropical Agriculture's (IITA) Cassava Breeding Unit in Ibadan, Nigeria. The roots of the various genotypes were washed thoroughly with tap water, peeled, and cut into chunks using a stainless-steel kitchen knife. A fresh sample of each genotype was portioned into two; one portion was packed in light-resistant sample bags and immediately analyzed for carotenoids ( $\beta$ -carotene profile) using HPLC. The second portion was oven-dried at 60°C for a period of 72 h, after which it was ground into flour using a Laboratory Hammer Mill (Perten 3102). Subsequently, the cassava flour samples were dispensed in sample containers, and kept in a 4°C refrigerator for the other analyses (tannins, total phenolics, total flavonoids, antioxidants, starch-hydrolysing enzymes, and pasting properties).

### Quantification of carotenoids by a reverse-phase HPLC

The carotenoid contents (total carotenoid and  $\beta$ -carotene profile) of the yellow-fleshed cassava genotypes were quantified using Maziya-Dixon et al. (2016) method. The total carotenoid content was determined using the spectrophotometric method, while the  $\beta$ -carotene profile [all-*trans*- $\beta$ -carotene ( $\beta$ C), 9-*cis*- $\beta$ -carotene (9-*cis*- $\beta$ C), and 13-*cis*- $\beta$ -carotene (13-*cis*- $\beta$ C)] was quantified using a reverse-phase HPLC system (Waters Corporation, Milford, MA, USA), comprising a binary HPLC pump (Waters 626), C30 YMC carotenoid column (4.6 × 250 mm, 3 µM), photodiode array detector (Waters 2996), auto-sampler (Waters 717), and Waters Empower 1 software. The carotenoids' chromatograms were acquired at 450 nm, and the identification and quantification of the individual  $\beta$ -carotenes were achieved by comparing their absorption spectrum and elution time with those of their corresponding pure external standards. To guard

against the degradation of the carotenoids induced by light, the analysis was conducted under subdued light, with the extracts collected into a vial wrapped with an aluminum foil.

## Preparation of cassava flour extract

The cassava flour extract was prepared by soaking 0.5 g of the cassava flour sample in 25 mL of methanol in an air-tight centrifuge tube of 50 mL capacity. After shaking the mixture for 1 h with a mechanical shaker, it was left for 12 h. After that, the mixture was centrifuged at 3,000 g for 10 min. The resulting supernatant (subsequently referred to as flour extract) was collected and kept at  $-4^{\circ}\text{C}$  until further analysis (Irondi et al., 2022a).

## Assay for tannins content

The procedure described by Joslyn (1970) was adopted to assay for the tannins content of the flour. A 0.5 g portion of the cassava flour was soaked for 15 min in 5 mL of acidified methanol (1% HCl in methanol), after which the mixture was vortexed and centrifuged for a period of 10 min at 3,000 g. Afterwards, 0.1 mL of the supernatant, distilled water (7.5 mL), Folin-Dennis reagent (0.5 mL), and  $\text{Na}_2\text{CO}_3$  solution (1 mL) were sequentially added and mixed. The volume of the reaction mixture was diluted to 10 mL by adding distilled water (0.9 mL). At 760 nm, the absorbance measurement was conducted following 30 min of incubation at room temperature. The tannin content of the flours was calculated using a tannic acid calibration curve and presented as tannic acid equivalent (TAE) in mg/g.

## Assay for total phenolics content

The protocol described by Elemosho et al. (2021) was adopted to quantify the total phenolic content of the cassava flours extract. This was achieved by dispensing 300  $\mu\text{L}$  of each extract into a test tube, to which Folin-Ciocalteu reagent (1.5 mL, 10 times dilution with distilled water) and  $\text{Na}_2\text{CO}_3$  solution (1.2 mL, 7.5% w/v) were sequentially added. The reaction mixture was vortexed and allowed to incubate at room temperature for 30 min before the absorbance was recorded at 765 nm in comparison to a control sample. For the blank preparation, distilled water (300  $\mu\text{L}$ ) was dispensed instead of the flours extract, with all other reagents added. The total phenolic level was computed using a standard curve of gallic acid and expressed in gallic acid equivalents (GAE) in mg per g.

## Assay for total flavonoids content

The protocol outlined by Meda et al. (2005) was followed to assay for the total flavonoids content of the cassava flours extract. To accomplish this, 0.5 mL of the extract, methanol (1.5 mL), 10% aluminum chloride (0.1 mL), 1M potassium acetate (0.1 mL), and distilled water (2.8 mL) were mixed in a test tube. Afterwards, the reaction mixture was vortexed and incubated for a period of 30 min (at room temperature), before the absorbance reading was taken at 514 nm. The total flavonoids content in the extract was computed using a quercetin standard curve and presented as quercetin equivalent (QE) in mg per g.

## Assay for 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) radical cation (ABTS<sup>+</sup>) scavenging capacity

The method described by Re et al. (1999) was adopted to assay for the ABTS<sup>+</sup> scavenging capacity of the cassava flours extract. First, a working reagent of ABTS<sup>+</sup> was prepared by mixing aqueous solutions of ABTS<sup>+</sup> (7 millimole/L) and  $\text{K}_2\text{S}_2\text{O}_8$  (2.45 millimole/L) in equal volumes. Then, for a total of 16 h, the mixture was incubated at room temperature in the dark. After that, the reagent's absorbance reading at 734 nm was adjusted to  $0.70 \pm 0.02$  using ethanol (95%). Next, an aliquot of 2.0 mL of the ABTS<sup>+</sup> working reagent and the flour extract (0.2 mL) were dispensed in a test tube, vortexed, and subjected to 15 min of incubation at room temperature in a dark condition. The absorbance reading was then taken at 734 nm, and the extract's ABTS<sup>+</sup> scavenging capacity was computed from a Trolox calibration curve and presented as Trolox Equivalent Antioxidant Capacity (TEAC) in millimole/g.

## Assay for 2,2-diphenyl-2-picrylhydrazyl radical (DPPH<sup>•</sup>) scavenging capacity

The procedure described by Cervato et al. (2000) was followed to assay for the DPPH radical scavenging capacity of the cassava flours extract. For this purpose, a mixture of 1.0 mL of different extract concentrations and 60 micromolar DPPH radical solution (3.0 mL) was subjected to incubation at room temperature in a dark condition for 30 min. Subsequently, the absorbance reading was taken at 517 nm in a spectrophotometer, and the DPPH radical scavenging capacity of the extract was computed and presented as  $\text{SC}_{50}$  (extract's concentration that scavenged DPPH<sup>•</sup> by 50%).

## Assay for ferric reducing power

The method described by Oyaizu (1986) was used to assay for the ferric reducing power of the cassava flour extract. This was achieved by incubating at  $50^{\circ}\text{C}$  for 20 min a mixture consisting of 2.5 mL each of the extract, 200 mM sodium phosphate buffer (pH 6.6), and 1% potassium ferricyanide. This was followed by the addition of 2.5 mL of 10% trichloroacetic acid to the mixture, which was then subjected to centrifugation for a period of 10 min at  $650 \times g$ . The supernatant was portioned into aliquots of 2.5 mL in different test tubes and distilled water (2.5 mL) and ferric chloride solution (1 mL, 0.1%) were sequentially added to each test tube. The reaction mixture was vortexed and the absorbance was subsequently read at 700 nm. The extract's ferric reducing power was expressed as gallic acid equivalent in mg per g.

## Assay for alpha-amylase inhibitory activity

An assay for the cassava flour extract's ability to inhibit  $\alpha$ -amylase activity was performed using the procedure outlined by Kwon et al. (2008). For this purpose,  $\alpha$ -amylase (EC 3.2.1.1) from porcine pancreas and its substrate (soluble starch) were used. A mixture of different dilutions of flour extract totaling 500  $\mu\text{L}$  and 500  $\mu\text{L}$  of  $\alpha$ -amylase (0.5 mg/mL, prepared in 0.02 mole/L sodium phosphate buffer, pH 6.9, containing 0.006 M NaCl) was subjected to incubation for 10 min at  $37^{\circ}\text{C}$ . Afterwards, starch solution (500

$\mu\text{L}$ , 1% in 0.02 mole/L sodium phosphate buffer) was added to the mixture, which was incubated for a period of 15 min at 37°C. After that, the reaction was stopped by adding 1.0 mL of the DNSA color reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 mole/L NaOH). The reaction mixture was then diluted with 10 mL of distilled water, after incubating in a boiling water bath for a period of 5 min and cooled to a room temperature. The absorbance was read at 540 nm. The percentage of  $\alpha$ -amylase inhibition was determined using the following equation:

$$\% \text{ Inhibition} = \left[ \frac{AR - AS}{AR} \right] \times 100$$

Where *AR* is the mean absorbance reading of the reference; *AS* is the mean absorbance reading of the sample.

### Assay for $\alpha$ -glucosidase inhibitory activity

An assay for the ability of the cassava flour extract to inhibit  $\alpha$ -glucosidase activity was performed as per the procedure outlined by Kim et al. (2005), in which  $\alpha$ -glucosidase (EC 3.2.1.20) from *Bacillus stearothermophilus* and its substrate, *para*-nitrophenylglucopyranoside (PNPG) were used. In this assay,  $\alpha$ -glucosidase (5 units) was incubated with the flour extract (20 g/mL) for a period of 15 min. Following that, 3 millimole/L PNPG in 20 millimole/L phosphate buffer (pH 6.9) was added to initiate the hydrolytic reaction for 20 min, during incubation at 37°C. The hydrolytic reaction was terminated by dispensing 2 mL of 0.1 M  $\text{Na}_2\text{CO}_3$  into the mixture. The absorbance reading of the yellow *p*-nitrophenol formed during PNPG hydrolysis was taken at 400 nm. The inhibition of  $\alpha$ -glucosidase was calculated thus:

$$\% \text{ Inhibition} = \left[ \frac{AR - AS}{AR} \right] \times 100$$

Where *AR* is the mean absorbance reading of the reference; *AS* is the mean absorbance reading of the sample.

### Analysis of pasting properties

The cassava flour samples' pasting characteristics (peak, breakdown, trough, final, set back viscosities, peak time, and pasting temperature) were analyzed using a Perten Scientific instrument's Rapid Visco Analyzer (RVA) (RVA-4500, Springfield, IL) (Ironi et al., 2022b). The RVA was attached to a Personal computer (PC) running Thermocline software. The pasting properties of the flour suspension (3.45 g of flour in 25 mL of distilled water) were read on the PC through the Thermocline software. The assay was performed at a 160 rpm constant stirring rate as per the following protocol: a 5 min holding period at 25°C; a 5°C/min heating rate between 25 and 95°C; a 5 min holding period at 95°C; a 5°C/min cooling rate to 25°C; and a 5 min holding period at 25°C. Viscosity was expressed in Rapid Visco Analyzer units (RVU); peak time was in minutes, while pasting temperature was expressed in °C.

## Statistical analysis of data

Data from replicate experiments (mean  $\pm$  standard deviation) were analyzed using one-way ANOVA and Duncan multiple range test (DMRT) at a 95% confidence level, using the 17th version of SPSS statistical software. The correlations between the bioactive constituents, antioxidant activity, and starch-hydrolysing enzymes inhibitory capacity of the samples were established by the Pearson correlation analysis.

## Results

### Carotenoids profile of 18 elite yellow-fleshed cassava genotypes

The carotenoid profile (on dry weight base) of the 18 elite yellow-fleshed cassava genotypes is presented in Table 1. The level of 13-*cis*- $\beta$ -carotene ranged from 1.26  $\mu\text{g/g}$  in IITA-TMS-IBA070337 to 5.11  $\mu\text{g/g}$  in IITA-TMS-IBA183044; *trans*- $\beta$ -carotene ranged from 2.37 to 19.76  $\mu\text{g/g}$  in IITA-TMS-IBA070337 and IITA-TMS-IBA183023; 9-*cis*- $\beta$ -carotene ranged from 1.38  $\mu\text{g/g}$  in IITA-TMS-IBA070337 to 6.68  $\mu\text{g/g}$  in IITA-TMS-IBA182993. The total  $\beta$ -carotene content ranged from 5.01  $\mu\text{g/g}$  in IITA-TMS-IBA070337 to 29.34  $\mu\text{g/g}$  in IITA-TMS-IBA183037, while the total carotenoids ranged from 6.75  $\mu\text{g/g}$  in IITA-TMS-IBA070337 to 45.88  $\mu\text{g/g}$  in IITA-TMS-IBA182962.

### Polyphenolic constituents of 18 elite yellow-fleshed cassava genotypes

The polyphenolic constituents of the 18 elite yellow-fleshed cassava genotypes are presented in Table 2. Tannins content ranged from 0.01 TAE mg/g in IITA-TMS-IBA183023 to 0.05 TAE mg/g in IITA-TMS-IBA183008; total phenolics content ranged from 0.77 GAE mg/g in IITA-TMS-IBA070593 to 2.06 GAE mg/g in IITA-TMS-IBA182959, and total flavonoids ranged from 0.27 QE mg/g in IITA-TMS-IBA070593 to 0.60 QE mg/g in IITA-TMS-IBA183037. As presented in the Table, the level of total phenolic in IITA-TMS-IBA182959 was significantly higher ( $p < 0.05$ ) than those of the other genotypes.

### Antioxidant activity of 18 elite yellow-fleshed cassava genotypes

The antioxidant activity of the 18 elite yellow-fleshed cassava flours is presented in Table 3. The reducing power ( $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ ) of the flours ranged from 1.32 GAE mg/g in IITA-TMS-IBA070337 to 2.59 GAE mg/g in IITA-TMS-IBA183001. The ABTS<sup>+</sup> scavenging capacity ranged from 9.94 TEAC mmol/g in IITA-TMS-IBA070593 to 15.59 TEAC mmol/g in IITA-TMS-IBA182986, and the DPPH<sup>•</sup>  $\text{SC}_{50}$  ranged from 14.56 mg/mL (strongest) in IITA-TMS-IBA183001 to 49.89 mg/mL (weakest) in IITA-TMS-IBA070593. There were significant variations ( $p < 0.05$ ) in the antioxidant activity of the flours of the 18 cassava genotypes studied.



TABLE 1 Carotenoids profile of 18 elite yellow-fleshed cassava genotypes (on a dry weight basis).

Accession no.	13- <i>cis</i> - $\beta$ C ( $\mu$ g/g)	<i>trans</i> - $\beta$ C ( $\mu$ g/g)	9- <i>cis</i> - $\beta$ C ( $\mu$ g/g)	Total $\beta$ C ( $\mu$ g/g)	Total carotenoids ( $\mu$ g/g)
070337	1.26 + 0.16 <sup>a</sup>	2.37 + 0.19 <sup>a</sup>	1.38 + 0.18 <sup>a</sup>	5.01 + 0.51 <sup>a</sup>	6.75 + 0.80 <sup>a</sup>
070593	3.27 + 0.65 <sup>bcd</sup>	5.98 + 0.94 <sup>b</sup>	3.29 + 0.88 <sup>b</sup>	12.53 + 2.47 <sup>b</sup>	20.59 + 3.35 <sup>b</sup>
182924	3.63 + 0.10 <sup>cdef</sup>	18.71 + 0.62 <sup>gh</sup>	4.09 + 0.15 <sup>bcd</sup>	26.42 + 0.76 <sup>defg</sup>	32.88 + 4.45 <sup>d</sup>
182926	3.98 + 0.26 <sup>defg</sup>	15.90 + 0.96 <sup>de</sup>	4.82 + 0.38 <sup>fgh</sup>	24.70 + 1.60 <sup>de</sup>	33.71 + 2.88 <sup>de</sup>
182959	4.62 + 0.30 <sup>gh</sup>	15.27 + 1.54 <sup>d</sup>	5.29 + 0.22 <sup>gh</sup>	25.18 + 1.58 <sup>def</sup>	36.12 + 3.92 <sup>defg</sup>
182961	4.07 + 1.02 <sup>efg</sup>	15.78 + 3.95 <sup>de</sup>	4.73 + 1.22 <sup>efgh</sup>	24.58 + 6.19 <sup>de</sup>	39.96 + 2.95 <sup>fg</sup>
182962	4.51 + 0.74 <sup>gh</sup>	19.18 + 1.60 <sup>gh</sup>	5.64 + 0.78 <sup>h</sup>	29.33 + 3.09 <sup>g</sup>	45.88 + 3.22 <sup>h</sup>
182963	3.65 + 0.04 <sup>cdef</sup>	16.90 + 1.59 <sup>defg</sup>	4.30 + 0.14 <sup>cdef</sup>	24.85 + 1.52 <sup>def</sup>	38.44 + 3.66 <sup>efg</sup>
182984	2.76 + 0.19 <sup>b</sup>	17.69 + 0.08 <sup>defgh</sup>	3.63 + 0.23 <sup>bcd</sup>	24.08 + 0.47 <sup>de</sup>	34.19 + 1.02 <sup>de</sup>
182986	3.30 + 0.14 <sup>bcd</sup>	16.19 + 1.37 <sup>def</sup>	3.86 + 0.29 <sup>bcd</sup>	23.35 + 1.66 <sup>d</sup>	33.52 + 1.37 <sup>de</sup>
182993	5.07 + 0.45 <sup>h</sup>	15.86 + 1.36 <sup>de</sup>	6.68 + 0.87 <sup>i</sup>	27.60 + 2.68 <sup>efg</sup>	37.27 + 3.69 <sup>defg</sup>
183001	3.20 + 0.10 <sup>bcd</sup>	10.07 + 0.58 <sup>c</sup>	3.74 + 0.25 <sup>bcd</sup>	17.00 + 0.73 <sup>c</sup>	25.85 + 1.35 <sup>c</sup>
183007	3.71 + 0.44 <sup>cdef</sup>	17.29 + 1.16 <sup>defgh</sup>	4.17 + 0.12 <sup>bcd</sup>	25.16 + 1.70 <sup>def</sup>	41.14 + 2.32 <sup>g</sup>
183008	3.90 + 0.68 <sup>defg</sup>	15.12 + 0.60 <sup>d</sup>	4.83 + 0.80 <sup>fgh</sup>	23.84 + 2.06 <sup>de</sup>	35.42 + 3.71 <sup>def</sup>
183023	2.98 + 0.10 <sup>bc</sup>	19.76 + 1.26 <sup>h</sup>	3.47 + 0.33 <sup>bc</sup>	26.20 + 1.67 <sup>defg</sup>	36.08 + 2.31 <sup>defg</sup>
183028	3.39 + 0.14 <sup>bcd</sup>	18.10 + 0.60 <sup>efgh</sup>	4.55 + 0.22 <sup>defg</sup>	26.03 + 0.84 <sup>defg</sup>	35.57 + 2.46 <sup>def</sup>
183037	4.10 + 0.26 <sup>fg</sup>	19.63 + 0.77 <sup>h</sup>	5.61 + 0.39 <sup>h</sup>	29.34 + 0.73 <sup>g</sup>	40.19 + 1.43 <sup>fg</sup>
183044	5.11 + 0.19 <sup>h</sup>	18.21 + 0.54 <sup>efgh</sup>	5.64 + 0.23 <sup>h</sup>	28.96 + 0.81 <sup>fg</sup>	38.67 + 2.32 <sup>efg</sup>

Values are mean  $\pm$  SD of triplicate assays. Values with different lowercase superscript letters along the same column vary significantly ( $p < 0.05$ ). 13-*cis*- $\beta$ C: 13-*cis*- $\beta$ -carotene; *trans*- $\beta$ C: all *trans*- $\beta$ -carotene; 9-*cis*- $\beta$ C: 9-*cis*- $\beta$ -carotene;  $\beta$ C:  $\beta$ -carotene. IITA-TMS-IBA precedes each accession number.

## Starch-hydrolysing enzymes inhibitory activity of 18 elite yellow-fleshed cassava genotypes

The inhibitory activity of the samples against the starch-hydrolysing enzymes (alpha-amylase and alpha-glucosidase) is presented in Table 4 as IC<sub>50</sub> values (extract's concentration that produced 50% inhibition of the enzyme's catalytic activity). The IC<sub>50</sub> values against alpha-amylase and alpha-glucosidase ranged from 21.75 to 33.18 mg/mL in IITA-TMS-IBA070593 and IITA-TMS-IBA182963, respectively, and 23.06 mg/mL in IITA-TMS-IBA182961 to 40.64 mg/mL in IITA-TMS-IBA182986. The starch-hydrolysing enzymes inhibitory activity of the 18 genotypes were significantly different ( $p < 0.05$ ).

## Pasting properties of 18 elite yellow-fleshed cassava genotypes

Table 5 presents the pasting properties of the 18-elite yellow-fleshed cassava genotypes. The peak, trough, and breakdown viscosities (in RVU) ranged from 93.83 to 972.60 in IITA-TMS-IBA182993 and IITA-TMS-IBA182961, 15.25–373.0 in IITA-TMS-IBA182984 and IITA-TMS-IBA182959, and 63.17 to 599.6 in IITA-TMS-IBA18299 and

IITA-TMS-IBA182926, respectively. The final and setback viscosities (in RVU) ranged from 23.17 to 488.3 in IITA-TMS-IBA182984 and IITA-TMS-IBA182926, and 3.920 to 128.1 in IITA-TMS-IBA182993 and IITA-TMS-IBA182926, respectively. The peak time ranged from 3.53 to 4.40 min in IITA-TMS-IBA182993 and IITA-TMS-IBA182924, while the pasting temperature ranged from 73.50 to 80.80°C in IITA-TMS-IBA182984 and IITA-TMS-IBA183008, respectively. Generally, the pasting properties of the different genotypes were comparable ( $p > 0.05$ ).

## Correlations among the bioactive components, antioxidant, and starch-hydrolysing enzymes inhibitory activities of flours of 18 elite yellow-fleshed cassava genotypes

Table 6 shows a significant and positive correlation ( $p \leq 0.0001$ ,  $r = 0.951$ ) between the total  $\beta$ -carotene and total carotenoid levels of the 18 elite yellow-fleshed cassava genotypes. The total carotenoid content negatively correlated significantly with the DPPH· SC<sub>50</sub> ( $p \leq 0.05$ ,  $r = -0.495$ ). Total phenolic content correlated significantly with ABTS<sup>+</sup> ( $p < 0.0001$ ,  $r = 0.839$ ) and DPPH· SC<sub>50</sub> ( $p < 0.0001$ ,  $r = -0.870$ ). Tannins content was significantly correlated with ABTS<sup>+</sup> ( $p < 0.05$ ,  $r = 0.553$ ).

TABLE 2 Polyphenolics constituents of 18 elite yellow-fleshed cassava genotypes.

Accession no.	Tannins (TAE mg/g)	Total phenolics (GAE mg/g)	Total flavonoid (QE mg/g)
070337	0.02 + 0.00 <sup>d</sup>	1.04 + 0.01 <sup>b</sup>	0.52 + 0.03 <sup>g</sup>
070593	0.01 + 0.00 <sup>b</sup>	0.77 + 0.01 <sup>a</sup>	0.27 + 0.00 <sup>a</sup>
182924	0.02 + 0.00 <sup>fg</sup>	1.38 + 0.01 <sup>f</sup>	0.41 + 0.01 <sup>c</sup>
182926	0.02 + 0.00 <sup>fg</sup>	1.36 + 0.00 <sup>ef</sup>	0.57 + 0.02 <sup>ij</sup>
182959	0.03 + 0.00 <sup>jk</sup>	2.06 + 0.05 <sup>l</sup>	0.46 + 0.01 <sup>ef</sup>
182961	0.03 + 0.00 <sup>k</sup>	1.28 + 0.06 <sup>d</sup>	0.51 + 0.03 <sup>g</sup>
182962	0.04 + 0.00 <sup>l</sup>	1.63 + 0.02 <sup>h</sup>	0.58 + 0.02 <sup>jk</sup>
182963	0.01 + 0.00 <sup>c</sup>	1.41 + 0.06 <sup>f</sup>	0.46 + 0.02 <sup>ef</sup>
182984	0.02 + 0.00 <sup>de</sup>	1.28 + 0.04 <sup>d</sup>	0.44 + 0.03 <sup>de</sup>
182986	0.03 + 0.00 <sup>j</sup>	2.00 + 0.03 <sup>k</sup>	0.51 + 0.02 <sup>g</sup>
182993	0.02 + 0.00 <sup>efg</sup>	1.23 + 0.03 <sup>c</sup>	0.33 + 0.01 <sup>b</sup>
183001	0.02 + 0.00 <sup>i</sup>	1.99 + 0.05 <sup>k</sup>	0.46 + 0.01 <sup>ef</sup>
183007	0.02 + 0.00 <sup>h</sup>	1.67 + 0.01 <sup>h</sup>	0.53 + 0.01 <sup>gh</sup>
183008	0.05 + 0.00 <sup>m</sup>	1.74 + 0.02 <sup>i</sup>	0.51 + 0.01 <sup>g</sup>
183023	0.01 + 0.00 <sup>a</sup>	1.31 + 0.00 <sup>de</sup>	0.47 + 0.00 <sup>f</sup>
183028	0.02 + 0.00 <sup>d</sup>	1.50 + 0.00 <sup>g</sup>	0.42 + 0.01 <sup>cd</sup>
183037	0.02 + 0.00 <sup>def</sup>	1.83 + 0.02 <sup>j</sup>	0.60 + 0.01 <sup>k</sup>
183044	0.02 + 0.00 <sup>g</sup>	1.04 + 0.02 <sup>b</sup>	0.54 + 0.01 <sup>hi</sup>

Values (mean ± SD of triplicate assays) with different lowercase superscript letters along the same column vary significantly ( $p < 0.05$ ). GAE, gallic acid equivalent; TAE, tannic acid equivalent; QE, quercetin equivalent. IITA-TMS-IBA precedes each accession number.

Further, total flavonoids content was significantly correlated with alpha-amylase  $IC_{50}$  ( $p \leq 0.05$ ,  $r = -0.532$ ), while each of the total carotenoids ( $p \leq 0.001$ ,  $r = 0.710$ ) and total  $\beta$ -carotene ( $p \leq 0.001$ ,  $r = 0.724$ ) had a significant positive correlation with  $\alpha$ -glucosidase  $IC_{50}$ .

## Discussion

### Carotenoids profile of 18 elite yellow-fleshed cassava genotypes

Among the 18 elite yellow-fleshed cassava genotypes, IITA-TMS-IBA182962 had the highest ( $p < 0.05$ ) level of total carotenoids. Carotenoids, which are the pigments in yellow-fleshed cassava, were reported to have some desirable health-promoting activities such as antioxidant activities, immune function enhancement, mammary tumor growth suppression, prevention of blindness due to age-related macular degeneration, and cardiovascular diseases (Chew and Park, 2004; Ahmed et al., 2005; Elemosho et al., 2021; Kareem et al., 2022). The range of  $\beta$ -carotene level, the predominant carotenoid in yellow-fleshed cassava, quantified in this study is higher than the range (1.12–6.53  $\mu\text{g/g}$ ) reported by Odoemelam et al. (2020) for yellow root cassava. Similarly, when compared to the range of total carotenoids

TABLE 3 Antioxidant activity of 18 elite yellow-fleshed cassava genotypes.

Accession no.	Reducing power (GAE mg/g)	ABTS <sup>+</sup> scavenging ability (TEAC mmol/g)	DPPH <sup>•</sup> $SC_{50}$ (mg/mL)
070337	1.92 + 0.11 <sup>h</sup>	11.50 + 0.42 <sup>bc</sup>	40.13 + 0.38 <sup>j</sup>
070593	1.42 + 0.09 <sup>ab</sup>	9.94 + 0.25 <sup>a</sup>	49.89 + 0.92 <sup>k</sup>
182924	1.58 + 0.01 <sup>cde</sup>	13.22 + 0.32 <sup>efgh</sup>	33.46 + 1.98 <sup>hi</sup>
182926	1.32 + 0.01 <sup>a</sup>	13.24 + 0.32 <sup>efgh</sup>	34.81 + 2.53 <sup>i</sup>
182959	1.87 + 0.02 <sup>gh</sup>	14.85 + 0.03 <sup>i</sup>	17.13 + 0.78 <sup>b</sup>
182961	1.50 + 0.04 <sup>bcd</sup>	13.94 + 0.24 <sup>h</sup>	25.97 + 0.29 <sup>ef</sup>
182962	1.98 + 0.02 <sup>h</sup>	12.57 + 0.82 <sup>def</sup>	22.14 + 0.28 <sup>cd</sup>
182963	1.48 + 0.05 <sup>bc</sup>	13.09 + 0.80 <sup>efg</sup>	32.18 + 2.14 <sup>gh</sup>
182984	1.62 + 0.03 <sup>de</sup>	12.44 + 0.30 <sup>de</sup>	31.37 + 0.55 <sup>g</sup>
182986	1.51 + 0.09 <sup>bcd</sup>	15.59 + 0.11 <sup>i</sup>	14.95 + 0.54 <sup>a</sup>
182993	1.46 + 0.07 <sup>bc</sup>	13.66 + 0.50 <sup>gh</sup>	23.90 + 0.52 <sup>de</sup>
183001	2.59 + 0.07 <sup>i</sup>	15.40 + 0.20 <sup>i</sup>	14.56 + 0.38 <sup>a</sup>
183007	1.48 + 0.11 <sup>bc</sup>	13.38 + 0.44 <sup>fgh</sup>	21.20 + 0.97 <sup>c</sup>
183008	1.40 + 0.09 <sup>ab</sup>	15.48 + 0.78 <sup>i</sup>	24.97 + 0.26 <sup>ef</sup>
183023	1.48 + 0.16 <sup>bc</sup>	12.90 + 0.64 <sup>efg</sup>	23.86 + 0.82 <sup>de</sup>
183028	1.78 + 0.03 <sup>fg</sup>	12.04 + 0.32 <sup>cd</sup>	26.40 + 1.91 <sup>f</sup>
183037	1.69 + 0.01 <sup>ef</sup>	13.48 + 0.23 <sup>gh</sup>	24.46 + 1.06 <sup>ef</sup>
183044	1.59 + 0.06 <sup>cde</sup>	10.79 + 0.21 <sup>b</sup>	34.68 + 0.94 <sup>i</sup>

Values (mean ± SD of triplicate assays) with different lowercase superscript letters along the same column vary significantly ( $p < 0.05$ ).  $SC_{50}$ : concentration of extract that scavenged 50% of DPPH<sup>•</sup>; TEAC: trolox equivalent antioxidant capacity; GAE, gallic acid equivalent. IITA-TMS-IBA precedes each accession number.

level in fresh roots of sweet yellow cassava (2.64–14.15  $\mu\text{g/g}$ ) (Carvalho et al., 2012) and bitter yellow cassava (3.65–18.92  $\mu\text{g/g}$ ) cultivars (Oliveira et al., 2010), the total carotenoids level (6.75 to 45.88  $\mu\text{g/g}$ ) in the eighteen elite yellow-fleshed cassava genotypes is higher. These differences could be attributed abiotic and biotic factors, period of sample collection, and method of extraction variations. Other factors that may have contributed to the variations include differences in the sample variety, composition of farm soil, and climatic conditions, all of which have been documented to influence the concentrations and biological activities of plant secondary metabolites (Mpofu et al., 2006; Braga et al., 2014; Dusuki et al., 2020). Generally, we observed a variation in the  $\beta$ -carotene level in the 18 elite yellow-fleshed cassava genotypes. The observed variation may be attributed to differences in their genetic makeup (Ayetigbo et al., 2018). Beta-carotene is notable for its provitamin A activity. Although an adequate intake or the recommended dietary allowance for carotenoids is not yet established (Andarwulan et al., 2021), with a  $\beta$ -carotene range of 5.01 to 29.34  $\mu\text{g/g}$ , the 18 elite yellow-fleshed cassava genotypes may serve as a rich dietary source of vitamin A in human nutrition. Moreover, with the emergence of COVID-19,  $\beta$ -carotene was also reported to offer protection against the disease (Anand et al., 2022).

**TABLE 4** Starch-hydrolysing enzymes inhibitory activity of 18 elite yellow-fleshed cassava genotypes.

Accession no.	Alpha-glucosidase IC <sub>50</sub> (mg/mL)	Alpha-amylase IC <sub>50</sub> (mg/mL)
070337	33.74 ± 3.96 <sup>ab</sup>	22.98 ± 3.39 <sup>a</sup>
070593	34.26 ± 5.80 <sup>ab</sup>	21.75 ± 2.51 <sup>a</sup>
182924	36.94 ± 3.93 <sup>b</sup>	23.36 ± 3.11 <sup>a</sup>
182926	40.37 ± 5.35 <sup>b</sup>	26.99 ± 3.96 <sup>abc</sup>
182959	35.92 ± 6.08 <sup>b</sup>	23.43 ± 2.83 <sup>a</sup>
182961	23.06 ± 3.82 <sup>a</sup>	25.93 ± 3.96 <sup>abc</sup>
182962	34.21 ± 4.95 <sup>ab</sup>	25.40 ± 3.39 <sup>abc</sup>
182963	33.73 ± 4.95 <sup>ab</sup>	33.18 ± 3.25 <sup>c</sup>
182984	38.57 ± 4.53 <sup>b</sup>	24.09 ± 3.11 <sup>ab</sup>
182986	40.64 ± 6.5 <sup>b</sup>	23.02 ± 2.97 <sup>a</sup>
182993	33.42 ± 4.53 <sup>ab</sup>	25.61 ± 4.38 <sup>abc</sup>
183001	34.60 ± 5.23 <sup>ab</sup>	22.85 ± 3.25 <sup>a</sup>
183007	38.56 ± 3.39 <sup>b</sup>	23.43 ± 2.69 <sup>a</sup>
183008	34.22 ± 3.39 <sup>ab</sup>	21.93 ± 2.97 <sup>a</sup>
183023	33.90 ± 4.53 <sup>ab</sup>	31.81 ± 4.38 <sup>bc</sup>
183028	33.17 ± 5.52 <sup>ab</sup>	28.44 ± 3.82 <sup>abc</sup>
183037	35.06 ± 5.52 <sup>b</sup>	22.81 ± 2.97 <sup>a</sup>
183044	34.23 ± 2.97 <sup>ab</sup>	24.10 ± 3.11 <sup>ab</sup>

Values (mean ± SD of triplicate assays) with different lowercase superscript letters along the same column vary significantly ( $p < 0.05$ ). IC<sub>50</sub>: concentration of extract that inhibits enzyme activity by 50%. IITA-TMS-IBA precedes each accession number.

## Polyphenolic constituents of 18 elite yellow-fleshed cassava genotypes

Tannins and flavonoids, as phenolic compounds, possess antioxidant activities due to their redox properties. Thus, they act as singlet oxygen quenchers, reducing agents, and hydrogen donors (Chang et al., 2001). In addition to these mechanisms, Andarwulan et al. (2021) noted that flavonoids could indirectly scavenge free radicals by inducing key antioxidant enzymes that protect against oxidative stress and electrophilic toxicants, such as S-transferase glutathione and NAD(P)H quinone oxidoreductase. The tannins, total phenolics, and flavonoids levels of the 18 elite yellow-fleshed cassava genotypes examined in this research differ from the values reported by Adefegha et al. (2021) in roasted and sun-dried cassava flours. For example, whereas we detected a total phenolics level in the range of 0.77–2.00 mg/g in the yellow-fleshed cassava genotypes, Adefegha et al. reported 51.35 and 64.82 mg/g as the total phenolics level in roasted and sun-dried cassava flours, respectively. As explained earlier, the differences may be related to variations in various factors, including the sample variety, farm soil compositions, climatic conditions, and abiotic and biotic factors, which were reported to affect the levels and bioactivities of phytochemicals (Braga et al., 2014; Dusuki et al., 2020). Apart from their antioxidant activities, studies have shown that polyphenolics possess starch-digesting enzymes inhibitory activity, which they

exhibit through non-specific binding to the enzymes, thereby denaturing them (Villiger et al., 2015; Elemosho et al., 2021). Additionally, phenolic compounds were reported to possess anti-diabetic, anti-hypertensive and anti-obesity properties (Ironi et al., 2018).

## Antioxidant activity of flours of 18 elite yellow-fleshed cassava genotypes

The ability of the cassava flours to scavenge DPPH<sup>•</sup> (denoted by SC<sub>50</sub>) represents their free radical scavenging capacity. DPPH (a stable free radical) is converted to  $\alpha,\alpha$ -diphenyl- $\beta$ -picryl hydrazine when reacted with antioxidants. As reported in previous studies, a lower DPPH<sup>•</sup> SC<sub>50</sub> of a plant extract/bioactive compound represents a stronger free radical scavenging capacity (Ironi et al., 2018; Alamu et al., 2021). Thus, among the 18 elite yellow-fleshed cassava genotypes, IITA-TMS-IBA18298, with the least DPPH<sup>•</sup> SC<sub>50</sub>, had the strongest DPPH<sup>•</sup> scavenging capacity.

ABTS cation radical is more reactive than the DPPH radical. The reactions involving ABTS cation radicals require an electron transfer process as opposed to the DPPH reactions, which involve the transfer of a H atom (Srikanth et al., 2010). It is well-known that in the body, a preponderance of oxidants (free radicals and reactive oxygen species) over the antioxidant level leads to oxidative stress, a denominator in the pathogenesis of many diseases (Ironi et al., 2018, 2019). Taken together, the DPPH and ABTS free radicals scavenging capacities of the cassava genotypes suggest that they can serve as dietary sources for retarding free radicals generation, and mopping them up from the body, thereby mitigating oxidative stress (Alamu et al., 2021). Similarly, the reducing power (Fe<sup>3+</sup> to Fe<sup>2+</sup>) of the cassava genotypes indicates their potential to alleviate Fe<sup>2+</sup>-mediated hydroxyl radical (OH<sup>•</sup>) formation from hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Obboh et al., 2008) and lipids oxidation (Hsu et al., 2003), also guarding against oxidative stress.

The antioxidant activity values of flours of the 18 elite yellow-fleshed cassava genotypes in this study differ from those previously reported in cassava flour. For instance, Ironi et al. (2019) reported a DPPH SC<sub>50</sub> of 0.48 ± 0.06 mg/mL and an ABTS cation radical scavenging capacity of 120.55 ± 1.7  $\mu$ mol TEAC/g in high-quality cassava flour made from the roots of white-fleshed cassava genotypes. Similarly, Adefegha et al. (2021) reported an ABTS cation radical scavenging capacity of 58.46 ± 0.33 and 99.92 ± 0.65 mmol TEAC/g for roasted and sun-dried cassava flours, respectively. As earlier stated, these variations may be due to genotypic, climatic conditions, and other abiotic and biotic factors differences, which affect phytochemicals' levels and, consequently, their bioactivities, including antioxidant activity (Braga et al., 2014; Dusuki et al., 2020).

## Starch-hydrolysing enzymes inhibitory activity of flour extracts of 18 elite yellow-fleshed cassava genotypes

The capacity of plant/food extracts to inhibit starch-hydrolysing enzymes (alpha-amylase and alpha-glucosidase)

TABLE 5 Pasting properties of flours of 18 elite yellow-fleshed cassava genotypes.

Accession no.	Peak viscosity (RVU)	Trough viscosity (RVU)	Breakdown viscosity (RVU)	Final viscosity (RVU)	Setback viscosity (RVU)	Peak time (min)	Pasting temp (°C)
070337	620.86 ± 101.00 <sup>abc</sup>	267.57 ± 12.21 <sup>bc</sup>	353.29 ± 89.35 <sup>a</sup>	328.81 ± 17.44 <sup>bc</sup>	61.25 ± 6.34 <sup>abc</sup>	4.18 ± 0.11 <sup>b</sup>	75.96 ± 0.61 <sup>ab</sup>
070593	627.85 ± 39.70 <sup>abc</sup>	221.81 ± 39.34 <sup>bc</sup>	406.04 ± 4.63 <sup>a</sup>	324.90 ± 20.75 <sup>bc</sup>	103.09 ± 18.94 <sup>c</sup>	4.18 ± 0.04 <sup>b</sup>	77.38 ± 1.14 <sup>ab</sup>
182924	647.35 ± 44.58 <sup>abc</sup>	291.42 ± 51.21 <sup>bc</sup>	355.94 ± 6.85 <sup>a</sup>	365.77 ± 11.73 <sup>bc</sup>	74.35 ± 39.54 <sup>c</sup>	4.25 ± 0.12 <sup>b</sup>	76.98 ± 1.54 <sup>ab</sup>
182926	738.67 ± 141.60 <sup>c</sup>	329.67 ± 26.65 <sup>c</sup>	409.00 ± 119.19 <sup>a</sup>	415.86 ± 58.91 <sup>c</sup>	86.19 ± 36.84 <sup>c</sup>	4.14 ± 0.27 <sup>b</sup>	76.93 ± 1.07 <sup>ab</sup>
182959	549.48 ± 6.53 <sup>abc</sup>	216.73 ± 49.42 <sup>bc</sup>	332.75 ± 45.58 <sup>a</sup>	289.06 ± 37.33 <sup>b</sup>	72.34 ± 13.05 <sup>bc</sup>	4.10 ± 0.09 <sup>b</sup>	75.56 ± 1.09 <sup>ab</sup>
182961	742.71 ± 154.22 <sup>c</sup>	316.06 ± 68.47 <sup>c</sup>	426.65 ± 122.15 <sup>a</sup>	370.90 ± 46.88 <sup>bc</sup>	54.83 ± 26.72 <sup>abc</sup>	4.02 ± 0.11 <sup>b</sup>	75.75 ± 0.98 <sup>ab</sup>
182962	567.27 ± 31.86 <sup>abc</sup>	261.60 ± 23.42 <sup>bc</sup>	305.67 ± 52.37 <sup>a</sup>	343.42 ± 30.65 <sup>bc</sup>	81.81 ± 8.15 <sup>c</sup>	4.24 ± 0.04 <sup>b</sup>	77.16 ± 0.79 <sup>ab</sup>
182963	540.67 ± 46.15 <sup>abc</sup>	229.98 ± 2.90 <sup>bc</sup>	310.69 ± 47.29 <sup>a</sup>	318.13 ± 6.94 <sup>bc</sup>	88.15 ± 5.97 <sup>c</sup>	4.18 ± 0.11 <sup>b</sup>	76.16 ± 1.33 <sup>ab</sup>
182984	364.40 ± 19.24 <sup>a</sup>	26.17 ± 15.72 <sup>a</sup>	338.23 ± 5.08 <sup>a</sup>	37.71 ± 20.22 <sup>a</sup>	11.54 ± 4.51 <sup>a</sup>	3.60 ± 0.06 <sup>a</sup>	73.90 ± 0.41 <sup>a</sup>
182986	631.46 ± 106.76 <sup>abc</sup>	269.71 ± 5.77 <sup>bc</sup>	361.75 ± 105.56 <sup>a</sup>	360.92 ± 10.27 <sup>bc</sup>	91.21 ± 9.36 <sup>c</sup>	4.03 ± 0.13 <sup>b</sup>	77.18 ± 1.58 <sup>ab</sup>
182993	376.96 ± 190.13 <sup>ab</sup>	47.46 ± 27.34 <sup>a</sup>	329.50 ± 183.82 <sup>a</sup>	60.69 ± 35.46 <sup>a</sup>	13.23 ± 8.60 <sup>ab</sup>	3.58 ± 0.04 <sup>a</sup>	74.33 ± 0.90 <sup>ab</sup>
183001	670.06 ± 14.39 <sup>bc</sup>	247.14 ± 17.34 <sup>bc</sup>	431.51 ± 8.69 <sup>a</sup>	325.07 ± 3.72 <sup>bc</sup>	92.24 ± 2.76 <sup>c</sup>	3.93 ± 0.01 <sup>b</sup>	75.98 ± 0.17 <sup>ab</sup>
183007	578.31 ± 23.17 <sup>abc</sup>	253.27 ± 45.73 <sup>bc</sup>	325.04 ± 24.64 <sup>a</sup>	328.31 ± 42.85 <sup>bc</sup>	75.04 ± 5.14 <sup>c</sup>	4.23 ± 0.06 <sup>b</sup>	76.54 ± 1.11 <sup>ab</sup>
183008	541.25 ± 96.52 <sup>abc</sup>	191.52 ± 37.46 <sup>b</sup>	349.73 ± 59.15 <sup>a</sup>	283.06 ± 70.97 <sup>b</sup>	91.54 ± 34.06 <sup>c</sup>	3.98 ± 0.11 <sup>b</sup>	78.00 ± 2.42 <sup>b</sup>
183023	662.07 ± 37.36 <sup>abc</sup>	245.94 ± 26.47 <sup>bc</sup>	416.13 ± 60.15 <sup>a</sup>	348.75 ± 15.91 <sup>bc</sup>	102.81 ± 10.66 <sup>c</sup>	3.92 ± 0.14 <sup>b</sup>	75.43 ± 1.13 <sup>ab</sup>
183028	639.36 ± 68.54 <sup>abc</sup>	252.46 ± 24.95 <sup>bc</sup>	386.90 ± 44.40 <sup>a</sup>	348.19 ± 27.04 <sup>bc</sup>	95.73 ± 3.90 <sup>c</sup>	4.07 ± 0.00 <sup>b</sup>	76.40 ± 0.44 <sup>ab</sup>
183037	624.73 ± 91.10 <sup>abc</sup>	249.35 ± 31.01 <sup>bc</sup>	375.38 ± 60.86 <sup>a</sup>	334.84 ± 42.93 <sup>bc</sup>	85.48 ± 15.7 <sup>c</sup>	4.13 ± 0.09 <sup>b</sup>	77.59 ± 0.64 <sup>ab</sup>
183044	706.31 ± 21.55 <sup>c</sup>	251.94 ± 28.53 <sup>bc</sup>	454.38 ± 7.60 <sup>a</sup>	351.59 ± 22.69 <sup>bc</sup>	99.65 ± 10.15 <sup>c</sup>	3.95 ± 0.03 <sup>b</sup>	75.99 ± 0.05 <sup>ab</sup>

Values are mean ± SD of triplicate assays. Values with different lowercase superscript letters along the same column vary significantly ( $p < 0.05$ ). IITA-TMS-IBA precedes each accession number.

has been used as an index of their potential for managing T2D (Ironi et al., 2021). Thus, the ability of the 18 elite yellow-fleshed cassava genotypes to inhibit alpha-amylase and alpha-glucosidase suggests that they may be of benefit for controlling postprandial hyperglycaemia characterizing T2D. Alpha-amylase and  $\alpha$ -glucosidase play important catalytic roles in hydrolysing dietary carbohydrates. Alpha-amylase is responsible in hydrolysing the  $\alpha$ -1,4 bonds of starch to form oligosaccharides such as malt-ose and dextrans, and disaccharides. On the other hand,  $\alpha$ -glucosidase found in the small intestine brush bor-der, completes the digestion of starch by hydrolysing the glycosidic bonds of the oligosaccha-rides and disaccharides, producing absorbable monosaccha-rides, including glucose and fructose (Tucci et al., 2010). Hence, inhibiting these two enzymes is a crucial mechanism of action of many anti-diabetic agents (Kim et al., 2005), including drugs, natural products, and functional foods.

The starch-hydrolysing enzymes inhibitory activity of the 18 elite yellow-fleshed cassava genotypes observed in this study could be as a result the phenolic compounds and carotenoids present in them. Previous studies revealed the ability of these two classes of phytochemicals to inhibit starch-digesting enzymes, thereby exhibiting anti-hyperglycaemic activity (Sugiura et al., 2015; Ironi et al., 2019; Elemosho et al., 2021).

## Pasting properties of flours of 18 elite yellow-fleshed cassava genotypes

According to Berski et al. (2011), pasting properties are important parameters used to determine the possible application of starch in the food industry. They represent the behavior of starch (changes in the physical and chemical structures) upon subjection under shear forces to a definite cycle of heating and cooling (Ironi et al., 2019). Peak viscosity, according to Alamu et al. (2017), denotes starch granules' ability to freely swell before their physical breakdown, which can be used to predict the final product quality of flour samples.

IITA-TMS-IBA182926 had the highest final viscosity among all the genotypes. The final viscosity is a measure of the cooked starch's stability and the paste's resistance to shear force during stirring (Maziya-Dixon et al., 2005). Thus, IITA-TMS-IBA182926 may be a good choice among all the genotypes for food and non-food products requiring high-viscosity starch. The stability of paste and its capacity to retrograde are assessed using setback viscosity (Liang and King, 2003). When the cooked paste cools to 50°C, the amylose and amylopectin components of the starch retrograde or reassociate, defining the viscosity of the cooked paste. The minimum



**TABLE 6** Correlations among the bioactive constituents, antioxidant and starch-hydrolysing enzymes inhibitory activities of flours of 18 elite yellow-fleshed cassava genotypes.

Parameters	TC (μg/g)	TβC (μg/g)	RP (GAE mg/g)	ABTS <sup>+</sup> (TEAC mmol/g)	DPPH <sup>·</sup> SC <sub>50</sub> (mg/mL)	Tannins (TAE mg/g)	TP (GAE mg/mL)	TF (QE mg/mL)	α-A IC <sub>50</sub> (mg/mL)
TβC (μg/g)	0.951****								
RP (GAE mg/g)	-0.251 <sup>ns</sup>	-0.273 <sup>ns</sup>							
ABTS <sup>+</sup> (TEAC mmol/g)	0.277 <sup>ns</sup>	0.242 <sup>ns</sup>	0.186 <sup>ns</sup>						
DPPH <sup>·</sup> SC <sub>50</sub> (mg/mL)	-0.495*	-0.448 <sup>ns</sup>	-0.379 <sup>ns</sup>	-0.820****					
Tannins (TAE mg/mL)	0.311 <sup>ns</sup>	0.197 <sup>ns</sup>	0.135 <sup>ns</sup>	0.553*	-0.440 <sup>ns</sup>				
TP (GAE mg/mL)	0.362 <sup>ns</sup>	0.322 <sup>ns</sup>	0.408 <sup>ns</sup>	0.839****	-0.870****	0.498*			
TF (QE mg/mL)	0.327 <sup>ns</sup>	0.289 <sup>ns</sup>	0.110 <sup>ns</sup>	0.249 <sup>ns</sup>	-0.335 <sup>ns</sup>	0.376 <sup>ns</sup>	0.404 <sup>ns</sup>		
α-A IC <sub>50</sub> (mg/mL)	-0.370 <sup>ns</sup>	-0.283 <sup>ns</sup>	-0.122 <sup>ns</sup>	-0.190 <sup>ns</sup>	0.312 <sup>ns</sup>	-0.281 <sup>ns</sup>	-0.165 <sup>ns</sup>	-0.532*	
α-G IC <sub>50</sub> (mg/mL)	0.710***	0.724***	-0.239 <sup>ns</sup>	0.172 <sup>ns</sup>	-0.344 <sup>ns</sup>	0.073 <sup>ns</sup>	0.266 <sup>ns</sup>	0.149 <sup>ns</sup>	-0.200 <sup>ns</sup>

\*Correlation is significant at  $p \leq 0.05$ ; \*\*\*correlation is significant at  $p \leq 0.001$ ; \*\*\*\*correlation is significant at  $p \leq 0.0001$ ; <sup>ns</sup>, correlation is not significant; TC, total carotenoids; TβC, total β-carotene; RP, reducing power; TEAC, trolox equivalent antioxidant capacity; SC<sub>50</sub>, concentration of extract that scavenged 50% of DPPH<sup>·</sup>; TAE, tannic acid equivalent; GAE, gallic acid equivalent; QE, quercetin equivalent; α-A, alpha-amylase; α-G, glucosidase; IC<sub>50</sub>, concentration of extract that inhibited 50% of enzyme activity.

temperature required to cook a starchy sample, according to [Offia-Olua \(2014\)](#), is the pasting temperature. Among all the genotypes, IITA-TMS-IBA182984 had the least pasting temperature, suggesting that the energy cost of developing products with it may be comparatively cheaper than those of the other genotypes.

## Correlations among the bioactive components, antioxidant and starch-hydrolysing enzymes inhibitory activities of flours of 18 elite yellow-fleshed cassava genotypes

The significant positive correlation between each of the total phenolics, tannin contents and the ABTS<sup>+</sup> scavenging capacity indicates that polyphenolics were majorly responsible for the ABTS<sup>+</sup> scavenging activity of the cassava genotypes. In contrast, a negative correlation between a bioactive compound and DPPH<sup>·</sup> SC<sub>50</sub> represents a stronger scavenging capacity since a lower DPPH<sup>·</sup> SC<sub>50</sub> denotes a more potent free radical scavenging activity ([Alamu et al., 2021](#); [Elemosho et al., 2021](#)). Thus, the negative correlations between each of total phenolics, total carotenoids, and DPPH<sup>·</sup> SC<sub>50</sub> suggest that the phenolics and carotenoids in the 18 elite yellow-fleshed cassava flours might have contributed synergistically to the DPPH radical scavenging capacity of the flours.

Among the bioactive constituents in the cassava flours, only total flavonoids had a significant negative correlation with α-amylase IC<sub>50</sub> ( $p < 0.05$ ;  $r = -0.532$ ). As with the DPPH<sup>·</sup> SC<sub>50</sub>, a lower IC<sub>50</sub> reflects a stronger enzyme inhibitory

capacity. Hence, flavonoids (a class of polyphenolics) contributed majorly to the α-amylase inhibitory activity of the cassava flours. This is consistent with an earlier report by [Elemosho et al. \(2021\)](#), who also suggested that phenolic compounds significantly contribute to the starch-hydrolysing enzymes inhibitory capacity of yellow-orange maize hybrids.

## Conclusions

Flours of the 18 elite yellow-fleshed cassava genotypes evaluated in this study were rich in carotenoids. They also contained polyphenolic compounds and exhibited antioxidant and starch-hydrolysing enzymes (alpha-amylase and alpha-glucosidase) inhibitory activities. Generally, the carotenoids and polyphenolics compositions of the different genotypes varied, but their starch-hydrolysing enzymes inhibitory and pasting properties were comparable. Correlation analysis revealed that total carotenoids and phenolics contents were associated with the antioxidant activity of cassava flour. In contrast, the total flavonoid was associated with their α-amylase inhibitory activity. Therefore, flours of the 18 elite yellow-fleshed cassava genotypes may serve as important dietary sources of antioxidants and starch-hydrolysing enzymes inhibitor, which may offer some benefits in oxidative stress and postprandial hyperglycaemic conditions.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

EAl and EI: conceptualization. EI, EAl, and BK: methodology. BK: investigation, software, and formal analysis. AA, BM-D, EP, EAl, and EAj: resources, review, and editing. BK and EI: writing—original draft preparation. EI, EAl, and EAj: supervision. EAl and AA: funding acquisition. EAl, AA, EP, and BM-D: project administration. All authors have read and agreed to the published version of the manuscript.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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