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Research article

Polyphenols in cassava leaves (*Manihot esculenta* Crantz) and their stability in antioxidant potential after *in vitro* gastrointestinal digestion

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ABSTRACT

The study was carried out to assess the effect of variety on polyphenols in cassava leaves and their stability in antioxidant activity before and after *in vitro* gastrointestinal digestion. The results showed that individual and total polyphenols content (TPC) and antioxidant activity of bound, free and bioaccessible polyphenols were significantly ($p < 0.05$) influenced by variety at harvesting maturity. The bound polyphenols had lower TPC (5.00–19.16 mg GAE/g) than free (39.16–89.61 mg GAE/g) throughout harvesting maturity. The polyphenols were strongly affected after *in vitro* digestion, however, salicylic, syringic and benzoic acids are the most bioaccessible. The free polyphenols of variety IRAD4115 had the highest value of FRAP (35.17 μg TE/g) at 12 months after planting (MAP), while, bound polyphenols showed the lowest DPPH (6.59 μg TE/g, variety EN at 12MAP). The antioxidant activity value evaluated by DPPH method was decreased significantly after *in vitro* gastrointestinal digestion. However, there was no significant difference between antioxidant activity of bioaccessible polyphenols (77.71 μg TE/g) and methanolic polyphenols (79.17 μg TE/g) assessed by FRAP method. These findings showed the stability of antioxidant potential of polyphenols in cassava leaves harvested at different periods after *in vitro* digestion. Thus cassava leaves harvested at appropriate maturity can be used as ingredient of functional food for nutraceutical benefits.

1. Introduction

A great portion of the World's population has malnutrition problems, which can be defined as a state of insufficient supply of quantitative and qualitative nutrients. Consequently, non-communicable diseases such as obesity, diabetes, cancer and cardiovascular diseases are becoming important diseases affecting the world population in the different regions. The vulgarisation of dietary diversities rich in nutrients, pigments and polyphenols which are excellent source of natural antioxidants may perhaps help populations to improve their life style. Many studies have shown that vegetables contain health improving compounds, and their consumption may thus help in the prevention of diseases (Gatto et al., 2016; Zhang et al., 2017). Despite the toxic cyanogen levels in cassava leaves, they are consumed as vegetable in various forms in the different parts of the World. The toxic potential of cassava leaf depending on the variety and plant age, hence, proper processing methods should be addressed to cassava leaf before its human consumption (Latif and Müller, 2015). The leaves can be harvested at any growth stages of plant, and many studies have proven that apart from being rich in nutritional composition, they have good concentrations of phytochemicals with high antioxidant activity (Koubala et al., 2015; Oresegun et al., 2016). It is reported that phenolics composition and antioxidant activity of vegetables are greatly affected by seasonal variation and environmental stress (Garcia-Diaz et al., 2018). The polyphenols in vegetables exist both as bound and free fraction (Sosulski et al., 1982; Chandrika et al., 2006; Zhang et al., 2017), and the bound fraction is reported to be more active than free fraction in grains (Kotaskova et al., 2016). The bound polyphenols may be released continuously in a slow manner through the gastrointestinal tract. Their abundant release in the gut during bacterial fermentation, can improve their bioaccessibility and bioavailability (Shahidi and Yeo, 2016). However, in the case of cassava leaves, there is no information about the differences in polyphenols composition in the bound, free fractions and bioaccessibility at different stages of maturity, and seasonal variation are not reported. The levels of bound and free polyphenols as well as their levels after *in vitro* gastrointestinal digestion of

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cassava leaves may be affected by the plant maturity, and varietal differences may also influence their antioxidant activity.

Therefore, the present study aimed to assess total polyphenol contents and *in vitro* antioxidant proprieties of bound and free fraction, and bioaccessible polyphenols of cassava leaves as affected by variety and harvesting maturity.

2. Materials and methods

2.1. Materials

Cassava (*Manihot esculenta* Crantz) leaves from five varieties such as EN and AD (local varieties), TMS92/0326 and TMS96/1414 from IITA (International Institute of Tropical Agriculture, Nigeria) (improved varieties) and IRAD4115 from IRAD (Institute of Agricultural Research for Development) in Adamawa region of Cameroon (improved variety) were used. Plants were grown in natural conditions in Tokombéré subdivision, the Far North Region of Cameroon, and their tender leaves were harvested in the morning during different plant maturity stages (months after planting, MAP), such as 6 MAP, 9 MAP, 12 MAP and 15 MAP. The cassava leaves were washed with tap water, cut into pieces and dried in an air oven (UN75 Memmert, 30–750, Germany) at 60 °C for 24 h. The dried samples were reduced into a powder, sieved and stored in opaque black polyethylene bag (Handgards®, Texas, USA) prior to analysis.

2.2. Chemicals and reagents

All standards were obtained from Sigma-Aldrich (Bengaluru, India). These standards were p-hydroxybenzoic, benzoic, gallic, vanillic, salicylic, protocatechuic, gentisic, syringic and Trolox. Other chemicals and reagents used were of analytical grade purchased from Sisco Research Laboratory (Bengaluru, India).

2.3. Free and bound polyphenolics extraction

Free and bound phenolic compounds were extracted following the method of Chen et al. (2017) with slight modifications. 20 ml of methanol was added into the fine powder (300 mg dry leaf sample) in triplicate for each analysis and mixed overnight by placing the test tube on shakers at room temperature (25 °C). The mixture was centrifuged at 10000 rpm for 20 min at 4 °C. The extraction solution was filtered with Whatman paper no.1. Then, the residue was washed two times using 5 ml of methanol and then centrifuged. The supernatants were combined and centrifuged in the same conditions as described above and filtered with Whatman paper no.1 to obtain the free polyphenol fraction. The solvent was evaporated at 45 °C to dryness and dissolved in methanol (HPLC grade) before being stored at -20 °C for subsequent uses. Concerning bound phenolics, the residue resulting from free phenolic extraction was mixed with 10 ml NaOH (2N) solution and boiled in shaking water bath at 50 rpm for 30 min before adding 5 ml HCl (2M) and then incubated for 60 min at 60 °C. The extraction mixture was cooled and 10 ml of ethyl acetate was added into the extraction solution. After 1 h shaking, the extraction solution was filtered and the ethyl acetate fraction was centrifuged under the same conditions as described above. The residue obtained after filtration was further mixed with 10 ml of ethyl acetate and centrifuged in the same conditions as described above before filtering through Whatman paper no.1. Finally, the combined supernatant was evaporated to dryness at 45 °C. The residue obtained was dissolved in methanol and centrifuged in the same conditions described previously to obtain the free polyphenol fraction. The methanol extract was kept at -20 °C until analysis.

2.4. In vitro gastrointestinal digestion and extraction of polyphenols

The method described by Ydjedd et al. (2017) with some changes was used. In short, the simulated solution was prepared in phosphate buffer (1M, pH 6.0). The digestion was started with the introduction of 1.5 mL of simulated salivary solution to both fraction (500 mg) containing 5 mg of porcine α-amylase (1000 U/mg). The pH of the mixture was adjusted to 7.0 before stirring for 10 min in a shaking water bath at 37 °C for 90 rpm. The next step consisted in adding 1.5 mL of the gastric digestion stimulating solution (10 mg/mL) consisting of pepsin (1:3000) initially diluted in HCl (0.1N). The pH was adjusted to 2 with HCl (6M) and the mixture was incubated at 37 °C in a shaking water bath (90 rpm) for 2 h. To simulate intestinal digestion, 2 mL of the intestinal solution (10 mg/mL) of pancreatin (100 U/mg) and 4 mg/mL of bile salts were added, respectively. The pH was adjusted to 7.0 with NaOH (1M), and the mixture was incubated for 3 h in a shaking water bath at a speed of 90 rpm. The enzymatic reaction was stopped by adding 1 mL of methanol. The supernatant was collected and after that 1 mL of methanol (HPLC grade) was added to the residue fraction then centrifuged at 4 °C at 10, 000 rpm for 10 min. After filtration through Whatman paper no.1, the solvent of combined supernatant was evaporated. The residue resulting was dissolved in methanol (HPLC grade) and filtered once with the 0.22 μm membrane (polypropylene, USA) and kept at -20 °C until the analysis.

2.5. Determination of total polyphenols

Bound, free and bioaccesible phenolics were determined using Folin Ciocalteu's reagent using microplate reader according to the protocol as described by Rocchetti et al. (2017) with some modifications. Briefly, 25 μL of standard or sample was mixed with 125 μL 0.2 M Folin-Ciocalteu reagent in a 96-well microplates. The microplate was covered with aluminium foil, and incubated for 10 min at room temperature. After incubation, 125 μL of 7.5 % sodium carbonate solution was added to each well. The microplate was shaken for 40 s and incubated for 40 min at room temperature before taking the absorbance at 765 nm using multimode microplate reader (Tecan SPARK 10M, V1.2.20, Austria). The standard curve plotted with gallic acid was used to evaluate the concentration of polyphenols.

2.6. Condition for HPLC-DAD analysis of individual phenolics

HPLC system (Hitachi Elite LaChrom, Hitachi High Technologies Japan, Tokyo, Japan) equipped with a Shodex C18-120-5 4E column, DAD detector and auto sampler, was used for quantification of phenolics. The C18 column (4.6 × 250 mm, 5 μm) (Supelco. Co) was used for separation of phenolics. System control and data acquisition were performed by Empower 3 Software (2010) (Corporation, Kyoto, Japan). Fractions were filtered with RC 0.45 μm (Phenex, USA) and filled in HPLC vials (Waters1.5 ml, USA). The mobile phases consisted of: (A) Milli-Q water containing 1 % acetic acid (v/v) and (B) methanol containing 1 % acetic acid (v/v). The column temperature was maintained at 30 °C and 20 μl of each sample was automatically injected. Elution was carried out at a flow rate of 1 ml/min. The linear gradient was formed as follows: 0–7 min (15 % B), 7–30 min (50 % B), 30–50 min (100 % B), 50–60 min (0 % B), 60–70 min (0 % B). Phenolics were monitored at 280, 320 and 360 nm. Peak of each phenolic was identified with authentic standard by comparing the retention time, and their quantification was done using standard curves.

2.7. Antioxidant activity assays

2.7.1. Ferric reducing antioxidant power (FRAP)

The FRAP was carried out according to the method described by Feregrino-Perez et al. (2008) with minor changes. Using a micropipette, 20 μL of the sample, blank or standard was added to 120 μL of the FRAP reagent in a 96 well microplates and the mixture was shaken for 50 s. The mixture was incubated at room temperature for 40 min. The absorbance was recorded at 595 nm with multimode reader (Tecan SPARK 10M, V1.2.20) and the results were determined using the Trolox standard and represented as Trolox Equivalent (TE).

2.7.2. 2.2-diphenyl-1-picyhydrazyl (DPPH)

The DPPH radical scavenging activity of the cassava leaves was carried out according to the method described by Feregrino-Perez et al. (2008) modified by Rocchetti et al. (2017) with some modifications. To the sample solution (40 µl), 150 µl of DPPH (1 mM) was added in a 96 well microplates. The solution was mixed for 40 s using microplate reader, and incubated for 40 min in the dark at 35 °C. After incubation, the absorbance was recorded at 517 nm with multimode reader (Tecan SPARK 10M, V1.2.20). The results were evaluated from the Trolox standard.

2.8. Statistical analysis

All data were analyzed by ANOVA using Statgraphics software (version. 16). Tukey's test was used to determine any significant difference between different varieties in the same month and in all harvest age for each variety and significance was accepted at level $p < 0.05$. The results were expressed as means ± standard deviation. All experiments were done in four replicates except individual phenolics.

3. Results and discussion

3.1. Effect of variety on total polyphenol content (TPC) of bound and free polyphenols of dry leaf without digestion

The TPC in free and bound polyphenol fractions of tender cassava leaves at different harvest maturity differed significantly ($p < 0.05$) for each variety (Table 1). For bound polyphenols, the TPC of variety EN (19.16 mg GAE/g) was significantly higher at 15 MAP compared to the others through the various harvests. However, the variety IRAD4115 (89.61 mg GAE/g) showed the highest concentration of TPC in free polyphenols at 12 MAP (Table 1). The lowest value of TPC in bound polyphenols was shown by variety TMS92/0326 (5.00 mg GAE/g) at 15 MAP which coincided with the rainy season. However, the results suggest that the accumulation of bound and free polyphenols may not only depend on varietal differences and season but also may be impacted by plant maturity. Similarly, some observations reported in literature indicated that the amounts of polyphenol in plants varied with respect to varieties and age maturity (Perez-Lopez et al., 2007). Zhang et al. (2017) also reported that the bound polyphenols contained lower polyphenols than free fraction in sweet corn, and the results of present study are consistent with the findings of Garcia-Diaz et al. (2018) who reported the significant effect of season variation on bioactive accumulation in beans.

Table 1. Effect of variety on total polyphenol contents (mg GAE/gdw) of bound and free polyphenols in cassava leaves of undigested sample (methanolic extract of dry leaf without digestion).

Harvest maturity	Varieties	Total polyphenol contents	
		Bound polyphenols	Free polyphenols
6MAP	TMS92/0326	15.14 ± 0.18^{aA}	40.57 ± 0.28^{dD}
	TMS96/1414	12.32 ± 0.23^{bB}	42.63 ± 0.82^{cD}
	IRAD4115	7.00 ± 0.27^{cD}	39.16 ± 1.51^{dD}
	EN	6.12 ± 0.46^{dD}	84.20 ± 0.87^{aA}
	AD	5.19 ± 0.13^{eD}	52.54 ± 0.07^{bB}
9MAP	TMS92/0326	10.52 ± 0.34^{aB}	41.91 ± 0.52^{dC}
	TMS96/1414	8.15 ± 0.56^{dcC}	77.23 ± 0.53^{bA}
	IRAD4115	8.35 ± 0.09^{dcB}	81.38 ± 2.86^{aB}
	EN	10.04 ± 0.28^{bB}	60.52 ± 1.52^{cB}
	AD	8.79 ± 0.39^{cC}	75.07 ± 0.46^{bA}
12MAP	TMS92/0326	8.96 ± 0.52^{dC}	47.17 ± 1.02^{cB}
	TMS96/1414	15.04 ± 0.38^{aA}	53.24 ± 1.14^{bC}
	IRAD4115	11.63 ± 0.29^{bA}	89.61 ± 0.87^{aA}
	EN	8.43 ± 0.35^{dC}	45.90 ± 1.67^{dD}
	AD	9.72 ± 0.26^{cAB}	44.09 ± 0.72^{dD}
15MAP	TMS92/0326	5.00 ± 0.27^{eD}	69.76 ± 0.79^{aA}
	TMS96/1414	7.58 ± 0.04^{dCD}	59.49 ± 0.79^{bB}
	IRAD4115	8.51 ± 0.29^{cBC}	70.64 ± 0.67^{aC}
	EN	19.16 ± 0.36^{aA}	57.19 ± 1.48^{cC}
	AD	10.13 ± 0.19^{bA}	51.27 ± 0.68^{dC}

MAP: month after planting; Cassava leaves harvested at 6MAP (onset dry season), 9MAP (main dry season), 12MAP (onset rainy season) and 15MAP (main rainy season); values represent means ± standard deviation of four replications (n = 4); Values are expressed in milligram (mg) gallic acid equivalent (GAE) per gram dry weight (gdw) of cassava leaves. Values followed by different lowercase letters in different variety in each column for the same month are significantly different ($p < 0.05$); values followed by different capital letters for each variety in each column for all month are significantly different ($p < 0.05$).

3.2. Total polyphenol contents (TPC) after in vitro gastrointestinal digestion influenced by variety

Figure 1 shows the TPC after *in vitro* gastrointestinal digestion of cassava leaves. The TPC values varied significantly between 39.42 and 298.32 mg/100g. These variations could be related to genetic characteristics of the varieties and seasons that may influence the concentration of bioaccessible polyphenols. However, bioaccessible polyphenols of the leaves of the variety IRAD4115 at 15 MAP is higher (Figure 1) as compared to the others. This result suggests that bioaccessibility could be influenced by phenol structure and solubilization (Williamson and Clifford, 2017; Blancas-Benitez et al., 2018). In addition, interactions of polyphenols with lipids, proteins and sugars could also affect the bioaccessibility (Karakaya, 2004; Jakobek, 2015). Whereas, methanolic TPC (Table 1) are higher than bioaccessible polyphenols: this can be explained by the presence of the insoluble fraction which is only excreted by microbial flora and fermentation in the large intestine for their high bioactivity (Zhao et al., 2018). However, there was a decrease of polyphenols after *in vitro* gastrointestinal digestion which was consistent with the results reported by Gunathilake et al. (2018) on six types of leaves.

3.3. Effect of variety on hydroxybenzoic acids of bound and free polyphenols of dry leaf without digestion

The highest value (660.67 µg/g) of p-hydroxybenzoic acid of bound fraction was exhibited by variety EN at 9 MAP, whereas, the free fraction of variety TMS96/1414 showed the highest concentration of p-hydroxybenzoic acid (1094.9 µg/g) at the same harvesting maturity (Table 2A). These results indicate that the p-hydroxybenzoic acid accumulated during dry season when plants may develop various mechanisms to fight against stress. The results obtained are similar to that observed by Yang et al. (2018) who reported that variation of p-hydroxybenzoic acid contents in bound and free polyphenols from barley varieties. The bound phenolics of variety TMS92/0326 showed the highest concentration (16.62 µg/g) at 6 MAP, while the highest value of p-catechuic acid in free fraction was found in variety EN (10.62 µg/g) at 12 MAP. The results are in agreement with Yang et al. (2018) who found a significant variation of p-catechuic acid in free and bound polyphenols of barley varieties. The variety EN (1.10 µg/g) showed the highest value of syringic acid at 12 MAP in bound fraction, while, the highest concentration of syringic acid from free phenolics was gotten in the AD variety (1.20 µg/g) at the same harvest maturity. The onset of rainy season was found to induce high syringic acid in both bound and free polyphenols as reported by Yang et al. (2018) who obtained a significant effect of variety on syringic acid of bound and free polyphenols from barley. Gallic acid was highly abundant in variety TMS92/0326 with the highest value for bound (1.72 µg/g) at 6 MAP and free phenolics (58.50 µg/g) at 15 MAP (Table 2A). The bound phenolics of TMS96/1414 variety (0.92 µg/g) and the free polyphenols of AD variety (0.13 µg/g) showed the lowest amount of gallic acid at 12 MAP. These results suggest that gallic acid concentration

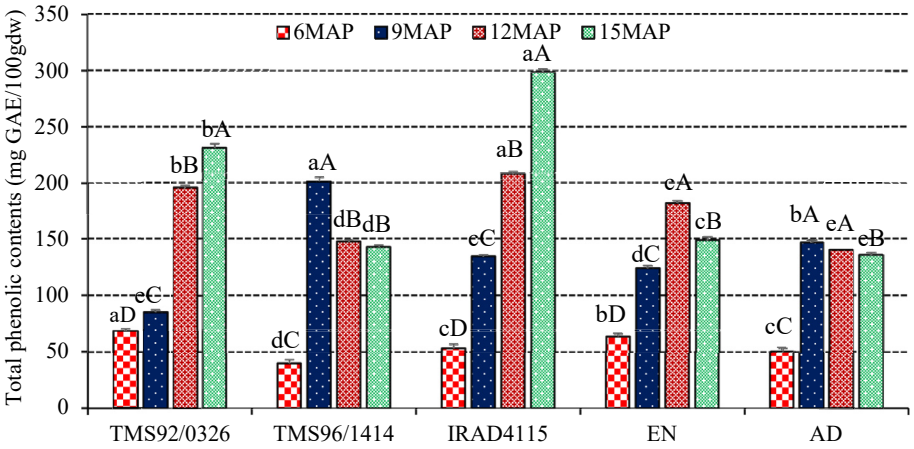

Figure 1. Effect of variety on bioaccessible polyphenolic contents (digested samples) of cassava leaves. MAP: month after planting; cassava leaves harvested at 6MAP (onset dry season), 9MAP (main dry season), 12MAP (onset rainy season) and 15MAP (main rainy season); Values represent means ± standard deviation of four replications (n = 4); Values are expressed in milligram (mg) per 100 g dry weight (dw). GAE: gallic acid equivalent; Bars followed by different lowercase letters in different variety for the same month are significantly different ($p < 0.05$); bars followed by different capital letters for each variety for all month are significantly different ($p < 0.05$).

varied depending on genetic differences of variety. Vanillic acid of bound and free phenolics varied significantly ($p < 0.05$), however, vanillic acid was not found in bound fractions of EN and AD varieties at 12 MAP. The bound phenolics of TMS92/0326 variety (1.27 μg/g) showed the highest concentration at 12 MAP and the free fraction of TMS92/0326 variety had the best concentration (585.91 μg/g) at 6 MAP. This result is in agreement with that reported by Suriano et al. (2018). The bound polyphenols of EN variety (0.21 μg/g) at 6 MAP and the free fraction at 12 MAP (20.25 μg/g) showed the highest concentrations of gentisic acid. The differences could be related to varietal differences mainly but the variation of environmental conditions also affect the phenolic accumulation. The bound polyphenols of AD variety (34.90 mg/g) and the free polyphenols of TMS96/1414 variety (265.93 mg/g) had the highest concentration of benzoic acid at 15 MAP, and benzoic acid was not detected in bound polyphenols of TMS92/0326, TMS96/1414, AD and EN varieties (Table 2B). This findings are in accordance with the results of Chen et al. (2014) who observed a great variation of benzoic acid in six ramie leaves. The bound fraction of AD variety (59.48 mg/g) had the highest concentration of salicylic acid at 6 MAP, while the IRAD4115 variety had the highest concentration (237.94 mg/g) at 15 MAP (Table 2B). These results suggest that salicylic acid was more accumulated during dry season and decreased during rainy season with respect to varietal differences. The results are similar with those reported by Suriano et al. (2018) on 20 barley genotypes.

3.4. Stability of hydroxybenzoic acids after in vitro gastrointestinal digestion (digested sample) impacted by the varieties

Table 3 shows a significant ($p < 0.05$) variation of bioaccessible hydroxybenzoic acids in leaves. The leaves of EN variety harvested at 9

Table 2. AEffect of cassava leaf variety of on hydroxybenzoic acids of bound and free phenolics fractions (methanolic extracts of dry leaf without digestion). Values are expressed in microgram per gram dry weight (μg/gdw) of leaves.

Harvest maturity	Var	p-Hydroxybenzoic acid (μg/gdw)		Protocatechiuc acid (μg/gdw)		Syringic acid (μg/gdw)		Gallic acid (μg/gdw)	
		Bound fraction	Free fraction	Bound fraction	Free fraction	Bound fraction	Free fraction	Bound fraction	Free fraction
6MAP	92	6.77 ± 2.15^{cC}	90.89 ± 9.06^{cbB}	16.62 ± 0.65^{aA}	3.47 ± 1.24^{aB}	0.18 ± 0.04^{bB}	0.60 ± 0.05^{cdBC}	1.72 ± 0.016^{aA}	4.25 ± 0.25^{dBCE}
	96	15.94 ± 1.84^{aC}	146.66 ± 3.67^{bCD}	0.15 ± 0.12^{cBC}	1.00 ± 0.59^{bD}	0.25 ± 0.01^{aB}	0.75 ± 0.07^{cC}	0.5 ± 0.05^{cB}	9.01 ± 0.66^{cC}
	IRA	6.19 ± 1.09^{cB}	47.48 ± 8.44^{cdD}	0.14 ± 0.58^{cD}	1.01 ± 0.67^{bC}	0.07 ± 0.01^{cC}	3.79 ± 0.77^{bC}	0.13 ± 0.01^{bA}	14.99 ± 1.30^{bD}
	EN	4.99 ± 0.83^{cdCD}	704.80 ± 89.69^{aA}	0.23 ± 0.11^{bC}	0.66 ± 0.39^{cD}	0.24 ± 0.01^{aC}	3.01 ± 0.10^{cdeC}	0.24 ± 0.01^{dC}	$9.23 \pm 0.38c^{BC}$
	AD	53.53 ± 7.37^{bB}	27.82 ± 2.99^{deD}	0.15 ± 0.79^{cB}	0.55 ± 0.30^{cB}	0.12 ± 0.00^{cC}	5.65 ± 0.25^{aB}	0.10 ± 0.00^{deC}	16.74 ± 0.74^{aA}
9MAP	92	121.88 ± 12.15^{bcA}	52.28 ± 7.14^{eD}	0.19 ± 0.22^{deD}	7.76 ± 3.52^{aA}	0.08 ± 0.01^{cC}	0.37 ± 0.06^{deCD}	0.2 ± 0.02^{deD}	26.71 ± 0.96^{dCB}
	96	180.55 ± 4.95^{bA}	1094.99 ± 59.95^{aA}	0.27 ± 0.17^{dB}	5.44 ± 3.91^{cA}	0.05 ± 0.00^{cdC}	6.63 ± 0.82^{bA}	0.03 ± 0.02^{dC}	78.80 ± 1559^{cB}
	IRA	42.50 ± 14.92^{dA}	368.51 ± 7.27^{cA}	0.54 ± 0.68^{cB}	0.94 ± 0.42^{eCD}	0.34 ± 0.12^{bB}	$9.50 \pm 0.67aA$	0.06 ± 0.00^{eC}	181.62 ± 7.43^{aA}
	EN	660.67 ± 91.29^{aA}	502.15 ± 87.84^{bB}	1.12 ± 0.89^{bA}	7.00 ± 1.12^{bB}	0.08 ± 0.02^{cD}	0.38 ± 0.06^{dC}	0.12 ± 0.06^{bA}	13.01 ± 0.58^{dcA}
	AD	31.88 ± 3.43^{deC}	346.94 ± 24.37^{dcA}	1.35 ± 1.00^{aA}	2.00 ± 5.60^{dA}	0.46 ± 0.03^{aA}	4.36 ± 0.64^{cC}	0.15 ± 0.03^{aA}	140.51 ± 6.12^{bB}
12MAP	92	10.12 ± 2.94^{cB}	113.53 ± 5.81^{cdA}	1.34 ± 0.53^{aB}	2.99 ± 0.21^{dCD}	0.60 ± 0.03^{bA}	0.97 ± 0.14^{dB}	0.14 ± 0.06^{aB}	51.55 ± 1.65^{bB}
	96	7.66 ± 0.93^{dcCD}	169.22 ± 20.81^{abC}	0.88 ± 0.11^{bC}	2.68 ± 0.98^{cB}	0.35 ± 0.07^{cA}	0.36 ± 0.02^{deCD}	0.92 ± 0.01^{deD}	7.72 ± 0.97^{cdC}
	IRA	3.51 ± 1.12^{deBC}	130.62 ± 7.67^{cB}	0.26 ± 0.15^{cC}	4.08 ± 2.01^{bA}	0.45 ± 0.04^{cA}	6.15 ± 0.49^{cB}	0.27 ± 0.02^{cD}	96.99 ± 19.80^{aB}
	EN	12.90 ± 2.66^{bcC}	52.68 ± 13.15^{eC}	0.61 ± 0.39^{bB}	10.62 ± 6.85^{aA}	1.10 ± 0.07^{aA}	12.01 ± 0.99^{aA}	0.58 ± 0.05^{bB}	8.69 ± 0.28^{cCD}
	AD	65.82 ± 3.05^{aA}	185.83 ± 12.69^{aB}	0.12 ± 0.06 dB	2.15 ± 0.80^{deBC}	0.8 ± 0.09^{dC}	7.99 ± 0.18^{bA}	0.13 ± 0.00^{dCD}	1.43 ± 0.06^{cdeD}
15MAP	92	6.82 ± 0.64^{cdC}	69.30 ± 6.09^{bC}	0.86 ± 0.07^{bC}	1.8 ± 0.23^{cdC}	0.05 ± 0.02^{dCD}	4.56 ± 0.50^{Abc}	0.89 ± 0.07^{abcC}	585.01 ± 71.59^{aB}
	96	104.22 ± 14.47^{aB}	930.11 ± 13.09^{aB}	1.01 ± 1.24^{aA}	2.14 ± 1.34^{bC}	0.28 ± 0.02^{cB}	5.17 ± 1.35^{bAB}	0.12 ± 0.09^{aA}	159.77 ± 7.05^{bA}
	IRA	6.06 ± 1.73^{cdeB}	86.23 ± 11.72^{bC}	0.72 ± 0.18^{bA}	3.83 ± 3.00^{aAB}	0.05 ± 0.02^{dCD}	1.31 ± 0.25^{dD}	0.75 ± 0.02^{cdB}	41.46 ± 1.45^{cC}
	EN	522.05 ± 66.24^{bB}	61.96 ± 8.28^{bC}	0.11 ± 0.08^{cD}	0.78 ± 0.57^{cC}	0.49 ± 0.09^{aB}	8.72 ± 1.09^{aB}	0.11 ± 0.013^{eB}	10.31 ± 1.34^{cdB}
	AD	15.92 ± 1.00^{cD}	126.06 ± 3.92^{bcC}	1.00 ± 0.02^{cC}	1.61 ± 0.16^{eBC}	0.39 ± 0.00^{bAB}	1.20 ± 0.11^{dD}	0.08 ± 0.01^{cB}	7.54 ± 0.97^{cdeC}

Var: varieties; MAP: month after planting; 92: TMS92/0326; 96: TMS96/1414; IRA: IRAD4115; cassava leaves varieties harvested at 6MAP (onset of dry season), 9MAP (main dry season), 12 (onset of rainy season) and 15MAP (main rainy season); values represent means ± standard deviation (n = 3). Values followed by different lowercase letters in different variety in each column for the same month are significantly different ($p < 0.05$); values followed by different capital letters for each variety in each column for all month are significantly different ($p < 0.05$).

MAP showed the highest value of biaoccessible gallic acid and salicylic acid, the leaves of the same variety and the same harvest maturity are more significantly bioaccessible (2798.49 μg/g). Similarly, the same leaves showed the highest value (6429 μg/g) of syringic acid (Table 3). These results indicate that varietal factors and harvest maturity influence the bioaccessibilty of hydroxybenzoic acids. The values of benzoic acid also varied significantly between 346.98 and 8142.42 μg/g for the leaves at 9 MAP and 6 MAP, respectively, whereas, for p-Hydroxybenzoic acid, the low bioaccessible content (9.63 μg/g) was observed at 9 MAP in leaves of TMS96/1414 variety and the significantly highest value (230.86 μg/g) was found in the leaves of the AD variety at 15 MAP. The highest bioaccessible gentisic acid was found in EN variety at 6 MAP as well as the highest value of 91.07 μg/g in bioaccessible vanillic acid (Table 3). These variations of the contents of bioaccessible hydroxybenzoic acids are attributed to the stability and instability of phenolics during the transition of gastrointestinal digestion and the affinity of digestive enzymes with phenolics (Gonzales et al., 2015; De Santiago et al., 2018), but also the initial concentration of these acids which have been reported by Gunathilake et al. (2018) on the bioaccessibility of phenolics of six leaves.

3.5. Effect of variety on antioxidant activities of undigested sample (methanolic extract of dry leaf without digestion)

3.5.1. Ferric reducing antioxidant power (FRAP)

FRAP of bound and free polyphenols varied significantly ($p < 0.05$) across the harvest maturity in different varieties (Table 4). The highest antioxidant activity throughout the harvest was shown by the IRAD4115variety at 12 MAP (35.17 μgTE/g in bound fraction and 79.17 μgTE/g in free fraction). This variation may be linked to the genetic factors of varieties, and the result is in accordance with Quartey et al. (2016) who observed variation of the total antioxidant among cassava leaves varieties, however, they did not report the bound and free polyphenols and effect of harvest maturity in their study.

3.5.2. 2.2-diphenyl-1-picyhydrazyl (DPPH)

The DPPH radical scavenging activity of bound polyphenols varied significantly during various harvest maturity according to the variety (Table 4). However, it was found that the bound polyphenols of EN variety (6.59 μgTE/g) had the best antiradical activity at 12 MAP, whereas free polyphenols of IRAD4115 variety at 6 MAP (9.99 μgTE/g) was found to possess the highest antioxidant activity as compared to the others. The variation in bound and free polyphenol contents (Table 1) during various harvest maturity may be responsible for these variations of antiradical activity, and these are in agreement with the results observed by Barkat et al. (2018) who reported the significant effect of harvesting time on DPPH radical scavenging activity in spinach. Furthermore, the variation could be due to the influence of plant maturity on antioxidant activity reported by Simao et al. (2013) for cassava varieties.

3.6. Stability of antioxidant activities of polyphenols after in vitro gastrointestinal digestion (digested sample) affected by the harvest maturity

3.6.1. Ferric reducing antioxidant power (FRAP)

The bioaccessibile polyphenols of the IRAD4115 variety at 12 MAP showed the significantly highest FRAP value (77.71 μgTE/g), while, the TMS96/1414 variety harvested at 6 MAP had the lowest value (30.51 μgTE/g) of FRAP (Table 4). These observations are similar to those reported by Bouayed et al. (2011). The results may be linked to the change in pH which could alter the structure of polyphenols, thus affecting antioxidant activity (Arenas and Trinidad, 2017). There was a slight decrease in antioxidant activity after in *vitro* gastrointestinal digestion of polyphenols that was consistent with observations reported by Tagliazucchi et al. (2010).

3.6.2. 2.2-diphenyl-1-picyhydrazyl (DPPH)

Cassava leaves of AD variety at 9 MAP (1.61 μgTE/g) had the strongest radical scavenging activity after *in vitro* gastrointestinal digestion (Table 4). The results may suggest the presence of multitudes of

Table 2B. Effect of cassava leaf variety on hydroxybenzoic acids of bound and free phenolics of undigested sample (methanolic extract of dry leaf without digestion) (next). Values are expressed in microgram per gram dry weight (μg/gdw), or milligram per gram dry weight (mg/gdw) of leaves.

Harvest maturity	Var	Vanillic acid (μg/gdw)		Gentisic acid (μg/gdw)		Benzoic acid (mg/gdw)		Salicylic acid (mg/gdw)	
		Bound fraction	Free fraction	Bound fraction	Free fraction	Bound fraction	Free fraction	Bound fraction	Free fraction
6MAP	92	0.04 ± 0.00^{eCD}	48.27 ± 1.30^{cdB}	0.06 ± 0.03^{cB}	0.83 ± 0.34^{cC}	10.48 ± 0.88^{aA}	3.53 ± 0.64^{bCD}	12.79 ± 2.17^{dA}	56.79 ± 1.59^{aA}
	96	0.37 ± 0.02^{bC}	585.91 ± 32.61^{aA}	0.04 ± 0.01^{cA}	1.12 ± 0.17^{cdCD}	7.46 ± 0.68^{bA}	3.69 ± 0.64^{bC}	23.55 ± 5.8^{cA}	69.64 ± 5.52^{bB}
	IRA	0.10 ± 0.00^{dC}	65.90 ± 1.73^{bcC}	0.03 ± 0.00^{dB}	7.01 ± 1.03^{bC}	0.35 ± 0.05^{dC}	89.07 ± 1.88^{aB}	3.87 ± 0.89^{eB}	54.16 ± 3.74^{bcD}
	EN	0.75 ± 0.05^{aA}	9.20 ± 0.33^{eB}	0.21 ± 0.02^{aA}	0.62 ± 0.26^{cC}	4.09 ± 0.39^{cB}	4.33 ± 0.12^{bD}	47.34 ± 6.94^{bA}	55.46 ± 2.83^{dD}
	AD	0.22 ± 0.02^{cC}	84.87 ± 2.10^{bB}	0.17 ± 0.02^{bA}	11.58 ± 3.51^{aA}	0.21 ± 0.05^{deC}	3.83 ± 0.18^{bA}	59.48 ± 0.25^{aA}	37.89 ± 1.46^{eD}
9MAP	92	0.98 ± 0.02^{aB}	21.34 ± 0.68^{dC}	0.12 ± 0.03^{aA}	1.26 ± 0.28^{dC}	0.63 ± 0.29^{dC}	12.44 ± 1.20^{cdB}	4.55 ± 0.33^{cC}	51.94 ± 6.30^{aB}
	96	0.56 ± 0.03^{cB}	89.83 ± 3.70^{bB}	0.05 ± 0.01^{bA}	9.84 ± 1.32^{bA}	0.69 ± 0.08^{dC}	77.78 ± 6.25^{bB}	8.57 ± 0.12^{eBCE}	24.10 ± 8.47^{C}
	IRA	0.70 ± 0.02^{bB}	137.48 ± 3.69^{aA}	0.06 ± 0.02^{bB}	13.19 ± 0.97^{aA}	9.22 ± 0.25^{aA}	120.69 ± 6.82^{aA}	6.50 ± 0.42^{bA}	33.39 ± 1.5^{dcB}
	EN	0.38 ± 0.03^{cB}	5.64 ± 0.34eC	0.06 ± 0.03^{bC}	0.85 ± 0.27^{deC}	3.53 ± 0.21^{cA}	14.15 ± 0.84^{cC}	7.52 ± 0.81^{aB}	18.21 ± 1.41^{bB}
	AD	0.72 ± 0.01^{bA}	64.89 ± 3.00cC	0.08 ± 0.02^{bcB}	7.06 ± 0.81^{cB}	3.83 ± 0.52^{bB}	3.90 ± 0.11^{eC}	2.55 ± 0.34^{dB}	23.78 ± 4.40^{bB}
12MAP	92	1.27 ± 0.03^{aA}	15.74 ± 0.29cD	0.11 ± 0.0^{bcAB}	2.55 ± 0.34^{dcB}	4.47 ± 0.20^{bB}	5.44 ± 0.60d^{C}	0.40 ± 0.05^{eD}	22.13 ± 8.98^{dD}
	96	0.60 ± 0.02^{cA}	12.71 ± 0.18^{dC}	0.06 ± 0.04^{bdA}	0.50 ± 0.07^{deC}	1.92 ± 0.25^{cB}	1.99 ± 0.27^{eCD}	1.09 ± 0.11^{dBC}	93.37 ± 3.80^{aA}
	IRA	0.73 ± 0.02^{bA}	88.86 ± 1.53^{bB}	0.09 ± 0.03^{bA}	11.37 ± 5.32^{bAB}	1.74 ± 0.18^{cdB}	82.55 ± 2.81^{aBC}	3.69 ± 0.22^{aB}	35.62 ± 8.98^{bA}
	EN	ND	9.51 ± 0.27^{cB}	0.17 ± 0.02^{aAB}	20.25 ± 1.71^{aA}	11.01 ± 0.25^{eA}	55.13 ± 1.94^{bB}	2.98 ± 0.17^{bBCE}	41.82 ± 4.51^{bB}
	AD	ND	120.99 ± 1.70^{aA}	0.02 ± 0.01^{dC}	7.16 ± 1.60b^{cB}	8.21 ± 0.55^{aA}	8.68 ± 0.83^{cB}	1.19 ± 0.06^{cC}	14.98 ± 1.50^{deD}
15MAP	92	0.07 ± 0.00^{dC}	72.04 ± 2.52^{cA}	0.09 ± 0.02^{dcAB}	7.72 ± 0.73^{bA}	ND	101.95 ± 3.31^{bA}	9.21 ± 0.43^{aB}	28.37 ± 0.48^{cbC}
	96	0.38 ± 0.02^{bC}	101.14 ± 1.13^{bB}	0.05 ± 0.03^{aA}	8.37 ± 1.47^{bB}	ND	265.93 ± 7.53^{aA}	1.32 ± 0.08^{dB}	46.28 ± 5.37^{bB}
	IRA	0.05 ± 0.00^{dD}	24.06 ± 0.34^{dD}	0.04 ± 0.00^{abB}	2.04 ± 0.38^{cCD}	ND	17.92 ± 2.19^{dD}	3.88 ± 0.26^{bcB}	237.94 ± 19.92^{aB}
	EN	0.14 ± 0.02^{cC}	174.87 ± 2.37^{aA}	0.08 ± 0.034^{aC}	14.15 ± 1.19^{aB}	ND	87.77 ± 0.45^{cA}	4.23 ± 0.21^{bBC}	37.39 ± 14.77^{cbBC}
	AD	0.56 ± 0.02^{aB}	7.77 ± 1.13^{eD}	0.06 ± 0.02^{aB}	1.83 ± 0.32^{dC}	34.90 ± 0.57^{aB}	11.49 ± 1.28^{deA}	0.89 ± 0.04^{deCD}	5.34 ± 7.57^{bA}

ND: not detected; Var: varieties; MAP: month after planting; 92: TMS92/0326; 96: TMS96/1414; IRA: IRAD4115; cassava leaves varieties harvested at 6MAP (onset of dry season), 9MAP (main dry season), 12 (onset of rainy season) and 15MAP (main rainy season); values represent means ± standard deviation (n = 3); Values followed by different lowercase letters in different variety in each column for the same month are significantly different ($p < 0.05$); values followed by different capital letters for each variety in each column for all month are significantly different ($p < 0.05$).

Table 3. Effect of cassava leaf on bioaccessibility of polyphenols of digested sample. Values are expressed in microgram per gram dry weight (μg/gdw) of leaves.

Harvest maturity	Variety	Gallic	Salicylic	Syringic	Benzoic	P-catechuic	p-Hydroxybenzoic	Gentisic	Vanillic
6MAP	92	7.01 ± 0.29^{dC}	118.42 ± 0.43^{cD}	295.89 ± 0.66^{eB}	2805.34 ± 2.14^{b}	352.22 ± 0.94^{cA}	33.08 ± 0.54^{dC}	4.46 ± 0.17^{cB}	7.45 ± 0.14^{dC}
	96	39.41 ± 0.41^{bA}	115.36 ± 0.66^{cD}	503.56 ± 0.74^{dA}	1661.62 ± 1.54^{c}	625.29 ± 2.17^{bA}	25.04 ± 0.68^{eC}	2.49 ± 0.29^{d}	17.83 ± 0.17^{cA}
	IRA	12.59 ± 0.64^{cC}	132.73 ± 0.30^{bC}	609.61 ± 0.24^{cA}	737.68 ± 2.96^{d}	185.92 ± 0.61^{eA}	183.71 ± 0.89^{bA}	0.88 ± 0.04^{eC}	21.68 ± 0.22^{bA}
	EN	48.14 ± 1.30^{aB}	37.42 ± 0.14^{dD}	2611.79 ± 0.81^{aB}	8142.41 ± 3.14^{a}	5021.89 ± 0.76^{aA}	53.29 ± 1.18^{cD}	66.58 ± 1.40^{aA}	91.07 ± 0.32^{aA}
	AD	11.83 ± 0.24^{cC}	148.05 ± 0.47^{aC}	648.57 ± 3.04^{bC}	693.93 ± 0.57^{e}	218.44 ± 0.62^{dB}	188.98 ± 0.91^{aA}	6.90 ± 0.21^{bA}	21.64 ± 0.27^{bB}
9MAP	92	13.80 ± 0.27^{dA}	1265.02 ± 1.01^{cA}	346.48 ± 0.37^{cA}	591.13 ± 1.39^{b}	130.98 ± 1.34^{bC}	119.52 ± 0.95^{cB}	3.44 ± 0.23^{aC}	5.30 ± 0.12^{cC}
	96	23.49 ± 0.39^{cB}	1558.22 ± 2.78^{bA}	278.04 ± 0.47^{dB}	458.73 ± 1.67^{c}	91.93 ± 0.59^{dD}	9.63 ± 0.16^{eD}	1.67 ± 0.07^{dB}	5.49 ± 0.20^{cC}
	IRA	30.94 ± 0.86^{bA}	493.42 ± 0.32^{eA}	219.13 ± 0.28^{eA}	348.24 ± 0.60^{d}	66.05 ± 0.62^{eC}	11.81 ± 0.11^{dB}	2.94 ± 0.02^{cC}	4.78 ± 0.29^{eC}
	EN	50.05 ± 1.27^{aA}	2798.49 ± 0.20^{aA}	6429.11 ± 0.43^{aA}	718.73 ± 3.64^{a}	141.45 ± 0.89^{aD}	141.59 ± 0.61^{bB}	1.43 ± 0.13^{eC}	16.19 ± 0.22^{aC}
	AD	9.67 ± 0.14^{eD}	510.73 ± 0.19^{dA}	783.63 ± 0.33^{bB}	346.98 ± 2.06^{d}	114.99 ± 0.53^{cD}	165.04 ± 0.35^{aB}	3.34 ± 0.04^{bB}	8.64 ± 0.11^{bC}
12MAP	92	11.20 ± 0.25^{dA}	203.46 ± 0.61^{bB}	163.68 ± 0.60^{dD}	615.27 ± 2.18^{c}	138.01 ± 1.06^{bC}	116.47 ± 0.32^{bB}	2.82 ± 0.02^{dC}	10.47 ± 0.18^{bB}
	96	30.12 ± 1.72^{bA}	201.46 ± 0.15^{bB}	188.30 ± 0.59^{bC}	833.13 ± 2.22^{a}	212.26 ± 0.21^{cB}	95.21 ± 0.25^{dA}	18.88 ± 0.26^{aA}	6.88 ± 0.11^{dC}
	IRA	19.49 ± 0.42^{cB}	217.89 ± 0.39^{aB}	146.05 ± 0.35^{eB}	526.36 ± 2.02^{e}	111.18 ± 1.07^{dB}	18.89 ± 0.12^{eB}	8.04 ± 0.61^{bB}	4.12 ± 0.07^{eC}
	EN	32.05 ± 2.61^{bD}	167.70 ± 0.42^{dB}	207.74 ± 0.30^{aD}	728.97 ± 1.63^{b}	211.32 ± 1.85^{cC}	124.38 ± 0.45^{aC}	2.81 ± 0.11^{dC}	13.17 ± 0.18^{aC}
	AD	49.66 ± 1.54^{aA}	192.16 ± 0.51^{cD}	178.82 ± 0.72^{cD}	600.89 ± 3.83^{d}	150.14 ± 0.71^{aC}	100.92 ± 0.47^{cC}	4.14 ± 0.12^{cA}	$8.21 \pm 0.19^{cC+}$
15MAP	92	9.62 ± 0.24^{eB}	193.11 ± 0.23^{bC}	213.05 ± 0.68^{cC}	1452.07 ± 1.62^{a}	219.59 ± 0.79^{cB}	165.13 ± 0.58^{bA}	55.64 ± 1.39^{aA}	16.21 ± 0.19^{cA}
	96	11.49 ± 0.12^{dC}	175.51 ± 0.45^{cC}	167.53 ± 0.25^{dD}	921.62 ± 1.59^{b}	162.93 ± 0.67^{eC}	81.05 ± 0.45^{cB}	3.41 ± 0.15^{dB}	11.50 ± 0.13^{dB}
	IRA	19.39 ± 0.42^{bB}	97.07 ± 0.18^{dD}	139.13 ± 0.45^{eC}	798.53 ± 1.50^{d}	186.09 ± 0.61^{dA}	12.61 ± 0.11^{dB}	4.67 ± 0.21^{cC}	6.67 ± 0.19^{eB}
	EN	41.17 ± 1.60^{aC}	50.58 ± 0.36^{eC}	861.39 ± 0.84^{bC}	801.83 ± 0.57^{c}	614.50 ± 0.65^{aB}	230.86 ± 0.91^{aA}	42.32 ± 0.59^{bB}	45.63 ± 0.28^{bB}
	AD	14.76 ± 0.44^{cB}	349.36 ± 0.09^{aB}	2670.83 ± 0.43^{aA}	421.81 ± 1.14^{e}	595.37 ± 0.88^{bA}	10.24 ± 0.01^{eD}	2.18 ± 0.04^{eB}	72.27 ± 0.40^{aA}

MAP: month after planting; 92: TMS92/0326; 96: TMS96/1414; IRA: IRAD4115; Cassava leaves harvested at 6MAP (onset dry season), 9MAP (main dry season), 12MAP (onset rainy season) and 15MAP (main rainy season); values represent means ± standard deviation (n = 3); gdw: gram dry weight. Values followed by different lowercase letters in different variety in each column for the same month are significantly different ($p < 0.05$); values followed by different capital letters for each variety in each column for all month are significantly different ($p < 0.05$).

Table 4. Effect of cassava leaf variety on FRAP and DPPH activity of bound and free fractions (undigested sample or leaves without digestion) and bioaccessible (digested sample) polyphenols. Values are expressed in microgram trolox equivalent per gram dry weight (μgTE/gdw) of leaves.

Harvest maturity	Varieties	FRAP (μgTE/gdw)			DPPH (μgTE/gdw)		
		Bound fraction	Free fraction	Bioaccessible	Bound fraction	Free fraction	Bioaccessible
6MAP	TMS92/0326	22.39 ± 0.77^{cA}	36.27 ± 0.38^{cD}	50.04 ± 0.13^{aD}	114.47 ± 0.52^{bB}	$176.67 \pm 2.8\ 3^{aA}$	9.87 ± 0.84^{bB}
	TMS96/1414	15.09 ± 0.30^{dC}	36.32 ± 0.65^{bD}	30.51 ± 0.22^{dD}	74.86 ± 1.51^{cB}	20.30 ± 0.12^{dCD}	5.99 ± 0.15^{cB}
	IRAD4115	33.48 ± 0.34^{aBC}	34.12 ± 1.68^{dD}	41.72 ± 1.70^{bB}	47.14 ± 1.33^{dA}	9.99 ± 0.21^{eCD}	10.79 ± 1.44^{bC}
	EN	24.76 ± 0.79^{bB}	51.11 ± 0.43^{aB}	41.74 ± 2.32^{bA}	147.25 ± 2.25^{aA}	86.15 ± 0.95^{bC}	11.49 ± 0.95^{aA}
	AD	21.59 ± 0.45^{cC}	31.41 ± 0.61^{eD}	35.09 ± 1.49^{cB}	14.16 ± 0.56^{eD}	61.63 ± 1.61^{cB}	9.18 ± 0.39^{bB}
9MAP	TMS92/0326	19.33 ± 0.35^{bB}	71.60 ± 0.17^{aA}	64.64 ± 2.09^{bB}	117.69 ± 1.78^{aB}	131.19 ± 1.03^{bB}	19.47 ± 3.38^{aA}
	TMS96/1414	19.45 ± 0.12^{bB}	69.76 ± 0.76^{cA}	68.56 ± 2.90^{aA}	21.34 ± 0.71^{dC}	132.46 ± 0.44^{bA}	3.88 ± 1.26^{cC}
	IRAD4115	18.68 ± 0.18^{cD}	72.78 ± 0.73^{aB}	39.24 ± 2.07^{cC}	14.19 ± 0.38^{eC}	70.69 ± 0.63^{cB}	4.40 ± 0.60^{cD}
	EN	34.68 ± 0.26^{aA}	60.25 ± 0.35^{cA}	36.77 ± 1.62^{cB}	64.70 ± 1.94^{bC}	169.59 ± 2.96^{aA}	7.83 ± 0.85^{bC}
	AD	18.84 ± 0.13^{dD}	70.00 ± 0.98^{bA}	38.36 ± 1.31^{cA}	35.31 ± 1.86^{cB}	70.79 ± 1.64^{cA}	1.61 ± 0.82^{dD}
12MAP	TMS92/0326	18.28 ± 0.44^{cD}	41.73 ± 0.89^{dC}	76.85 ± 2.19^{aA}	15.33 ± 0.41^{dC}	172.58 ± 2.31^{aA}	8.77 ± 2.52^{cC}
	TMS96/1414	32.93 ± 0.85^{bA}	47.83 ± 0.17^{bB}	51.89 ± 0.50^{bB}	78.07 ± 2.05^{aA}	20.92 ± 0.47^{dC}	9.87 ± 0.67^{cA}
	IRAD4115	35.17 ± 0.76^{aA}	79.17 ± 1.05^{aA}	77.71 ± 1.67^{aA}	42.92 ± 2.11^{bAB}	10.54 ± 0.69^{eC}	15.40 ± 3.14^{aB}
	EN	14.41 ± 0.366^{dD}	35.89 ± 0.08^{eD}	34.95 ± 0.89^{cC}	6.59 ± 0.29^{eD}	69.41 ± 0.76^{bD}	11.53 ± 0.58^{abA}
	AD	32.91 ± 0.84^{bA}	44.39 ± 0.46^{cC}	32.54 ± 1.53^{cB}	20.83 ± 0.40^{cC}	54.21 ± 1.42^{cC}	14.34 ± 1.85^{bA}
15MAP	TMS92/0326	19.25 ± 0.10^{dCB}	70.97 ± 0.51^{aAB}	58.09 ± 1.00^{aC}	154.56 ± 0.11^{aA}	84.40 ± 0.25^{bC}	20.91 ± 2.31^{aA}
	TMS96/1414	14.52 ± 0.13^{eCE}	45.85 ± 0.44^{dC}	41.92 ± 2.07^{bC}	16.73 ± 0.82^{dD}	24.23 ± 0.33^{dB}	5.98 ± 2.01^{cB}
	IRAD4115	33.61 ± 0.63^{aB}	65.42 ± 1.19^{bC}	39.24 ± 2.07^{bcC}	9.69 ± 0.52^{eD}	90.24 ± 0.25^{aA}	17.32 ± 2.15^{aA}
	EN	24.20 ± 0.13^{cBC}	39.55 ± 0.41^{eC}	36.77 ± 1.62^{cB}	69.16 ± 0.76^{bB}	90.28 ± 0.22^{aB}	9.21 ± 1.39^{bB}
	AD	29.45 ± 0.33^{bB}	48.21 ± 0.18^{cB}	38.37 ± 1.41^{cA}	$51.\ 13 \pm 1.34^{cA}$	45.22 ± 1.26^{cD}	$8.\ 76 \pm 2.87^{bC}$

FRAP: ferric reducing antioxidant power; DPPH: 2.2-diphenyl-1-picyhydrazyl; g dw: gram dry weight; μgTE: microgram trolox equivalent; MAP: month after planting; Cassava leaves harvested at 6MAP (onset dry season). 9MAP (main dry season). 12MAP (onset rainy season) and 15MAP (main rainy season); values represent means ± standard deviation of four replications (n = 4); Values followed by different lowercase letters in different variety in each column for the same month are significantly different ($p < 0.05$); values followed by different capital letters for each variety in each column for all month are significantly different ($p < 0.05$).

phenolics that act synergistically after *in vitro* gastrointestinal digestion to exhibit a strong antiradical activity, however, the variation in antioxidant activity could be linked to the maturity of the plant, whose sample matrix could influence the bioaccessibility of polyphenols with structures having a strong affinity for free radicals (Simao et al., 2013). The antiradical activity evaluated could be related only to the bioaccessible polyphenols, therefore the insoluble fraction may be more bioactive after fermentative action of the microbial flora (Williamson and

Clifford, 2017). This suggests that the present results are very important because bioaccessible polyphenols showed the strongest radical scavenging activity as compared to those of methanolic polyphenols from the native cassava leaf samples. These observations have also been reported by several authors (Bouayed et al., 2011; Gunathilake et al., 2018) in their studies on six types of leaves.

4. Conclusion

This finding clearly shows that variety and harvesting maturity significantly affect the polyphenol contents and antioxidant activity of cassava leaves before and after *in vitro* gastrointestinal digestion. The antioxidant activity of bound polyphenols showed significantly higher activity than the free fraction, however, there were some discrepancies throughout the harvest maturity among the varieties. The antioxidant activity was increased significantly after *in vitro* gastrointestinal digestion with some variability. The bioaccessible phenolics are stable and showed the highest activity compared to the methanolic extract of leaves with unexpected variability among varieties. These results suggest that cassava leaves can be considered as a good reliable source of natural polyphenols and bioaccessible antioxidant compounds throughout the harvest maturity that could be useful as functional food ingredients.

Declarations

Author contribution statement

Alphonse Laya: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Benoît B. Koubala: Conceived and designed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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