

## Original article

**Influence of bunch maturation and chemical precursors on acrylamide formation in starchy banana chips**Patchimaporn Udomkun,<sup>1\*</sup> Rony Swennen,<sup>2,3</sup> Cargele Masso,<sup>4</sup> Bhundit Innawong,<sup>5,6\*</sup> Apollin Fotso Kuate,<sup>4</sup> Amos Alakonya<sup>7</sup> & Bernard Vanlauwe<sup>8</sup>

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**Summary** The present study investigated the effect of ripening stages and chemical precursors on acrylamide formation in deep-fried chips of five plantains and one cooking banana. The highest level of acrylamide was found in the cooking banana, followed by False Horn plantain and French plantain, respectively. French plantain hybrids exhibited a significantly lower ( $P < 0.05$ ) level of acrylamide when compared to French plantain. The ripening stage demonstrated a positive Pearson correlation ( $P < 0.05$ ,  $r = 0.57$ ) with acrylamide formation. As ripening progressed, the levels of glucose and fructose significantly increased ( $P < 0.05$ ) and showed a positive correlation with acrylamide formation ( $r = 0.85$  and  $0.96$ , respectively). The level of the amino acid asparagine during ripening was not correlated with acrylamide formation. In contrast, the level of histidine, arginine, iso-leucine and cystine during ripening was positively correlated ( $P < 0.05$ ,  $r > 0.60$ ) with acrylamide formation in fried chips. The higher level of TP was significantly related ( $P < 0.05$ ) to the lower level of acrylamide ( $r = -0.62$ ). The reduced levels of carotenoid isomers, except lutein, during fruit ripening were positively correlated ( $P < 0.05$ ) with acrylamide formation, especially *trans*-BC ( $r = 0.72$ ) and *9-cis*-BC ( $r = 0.64$ ).

**Keywords** Acrylamide formation, atmospheric frying, chemical composition, plantain and cooking banana, plantain hybrid, ripening stage.

**Introduction**

Plantain (AAB subgroup), which is sweet acid and starchy, is an essential staple for more than 70 million people in sub-Saharan Africa. Other starchy bananas, like Bluggoe, belong to the ABB group (Eggleston *et al.*, 1992). The main plantain producers in the continent are small-scale farmers who grow the crop for their own consumption or local markets. In 2019, Ghana produced over 4.9 million tonnes of plantain, the highest globally, followed by the Democratic Republic of Congo, Cameroon and Nigeria (FAO-STAT, 2021), together comprising 60% of total

production. Plantain fruits are a good source of dietary carbohydrates, fibre, antioxidants (phenols), vitamins A and C and minerals K and Fe (Tribess *et al.*, 2009). Besides, plantain is recommended for diabetics due to its low glycaemic index value (Eleazu & Okafor, 2015). When ripe, the fruit can be eaten fresh for energy and is locally used in many different dishes, according to the ripening stage and sociocultural norms of each ethnic group (Honfo *et al.*, 2011). Green or unripe fruits are often boiled or processed as chips or flours (Anyasi *et al.*, 2015).

Deep-fat frying in palm oil is one of the main postharvest operations for preserving the quality of plantain in Africa, and the fried chip is a popular snack for both internal consumption and export. The

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process involves simultaneous transfers of mass (losing water and absorbing oil) and heat (Krokida *et al.*, 2000). During frying at a high temperature (150–200 °C), heat from the oil is transferred to the food surface by convection and continuously transferred into the centre by conduction (He *et al.*, 2013). Like other fried products, oil content and colour change are crucial organoleptic properties that affect acceptability and consumers' decision to buy fried plantain chips. Apart from the adverse effects due to excessive fat consumption, the frying process also leads to a high accumulation of acrylamide, especially on the surface of carbohydrate-based foods (Bassama *et al.*, 2011).

Acrylamide is classified as a potential carcinogen to humans (group 2A) due to its neurotoxic and genotoxic properties on the basis on animal studies (IARC, 1994). At elevated temperatures, the main mechanism in acrylamide formation is most likely due to the Maillard reaction between the asparagine amino acid and reducing sugars, notably glucose and fructose (Stadler *et al.*, 2002; Wang *et al.*, 2019) and/or from reducing sugars and other amino acids that can produce acrylic acid, such as  $\beta$ -alanine, aspartic acid, carnosine, cysteine and serine (Yaylayan *et al.*, 2005). Given the presence of amino acids and reducing sugars in a food, the formation of acrylamide can follow either a generic amino acid route (Nguyen *et al.*, 2016) or a specific route of asparagine (Parker *et al.*, 2012). The generic amino acid route involves a dicarbonyl intermediate. When reducing sugars react with any amino group, a Schiff base is formed and rearranges afterwards to give an Amadori product (in the case of an aldose) or a Heyns product (in the case of a ketose). These products dehydrate and fragment to form the highly reactive dicarbonyl compounds, deoxyosuloses and/or hydroxycarbonyl compounds. The dicarbonyl compounds subsequently react with asparagine via Strecker degradation, leading to the formation of acrylamide (Yaylayan & Stadler, 2005; Champrasert *et al.*, 2021). In the specific amino acid route, acrylamide is generated without rearrangement of the Amadori products and fragmentation of sugar (Nguyen *et al.*, 2016).

Other potential indirect routes to acrylamide formation have been also identified. For example, lipid oxidation pathway contributes to acrylamide formation in fried products (Gertz & Klostermann, 2002). In the presence of carbonyl groups, such as aldehydes and ketones, lipid oxidation breakdown products could react with asparagine to form acrylamide despite the absence of reducing sugars (Ehling & Shibamoto, 2005). This is in agreement with a study of Kuek *et al.* (2020) who indicated that secondary lipid oxidation constituents have resulted in a positive influence to acrylamide formation during intermittent frying of French fries. Apart from asparagine pathway,

Weishaar (2004) and Yaylayan & Stadler (2005) proposed minor pathway such as acrolein as the alternative route for acrylamide formation. The acrolein which can be formed by different pathways, including the oxidative degradation of lipids, could react further via acrylic acid to form acrylamide (Kuek *et al.*, 2020). Aspartic acid can also release acrylic acid without the involvement of sugars or a carbonyl source following a concerted decarboxylation/deamination pathway (Yaylayan & Stadler, 2005). The concentration of acrylamide depends on various determinant factors such as cultivar, soil characteristics and fertilisation, pre-treatment process, cooking temperature and time, the level of main precursors present in plant-derived foods and storage conditions (Friedman, 2015).

There are a few known studies for evaluating physical and chemical changes in plantain chips due to the frying process. A study by Ikoko & Kuri (2007) showed that oil intake, moisture content, total volume and frying time were reduced by osmotic dehydration pre-treatments, while there was an increase in colour parameters, texture peak force and rancidity after frying. Quayson & Ayernor (2007) reported that plantain chips contained a low level of acrylamide compared to other Ghanaian traditional foods derived from roots and tubers. The reduction of asparagine during postharvest ripening could contribute significantly to reducing acrylamide in plantain-based foods (Bassama *et al.*, 2011) when they evaluated acrylamide content in a plantain matrix during heating without additional precursors. Shamlal & Nisha (2017) investigated the effect of ripening on acrylamide formation in deep-fried plantain chips made from the Nendran variety (*Musa paradisiaca*). They observed that reducing total phenolic and total flavonoid content during ripening had a negative correlation with acrylamide formation. However, no studies on the effect of cultivar and ripening stage on nutrient composition and acrylamide formation in plantain chips have been reported. Therefore, this study investigated the effect of different cultivars (plantains, cooking banana and plantain hybrids) and ripening stages on the changes in the chemical composition of plantain and sought to determine their correlations with acrylamide formation in deep-fried plantain chips.

## Materials and methods

### Sample preparations

Two local plantain cultivars (Elat and Batard) were collected from Ntui, Cameroon (4°27'N, 11°37'E, 526 masl), while one cooking banana (Daru of the Bluggoe type) and the other three improved cultivars (PITA 14, PITA 21 and PITA 27) were harvested from a field plot at the IITA, Yaoundé research station, Cameroon

(3°21'N, 11°28'E, 624 masl). Elat has a French-type bunch, while Batard has a False Horn bunch. The French plantains are characterised by both male and female flowers when mature, whereas False Horn type bunches have only female flowers and much bigger fruits compared to French-type bunches. PITAs are plantain hybrids of French-type bunch which have resistance to black leaf streak disease and other pests.

In this study, fully developed bunches with deep green undamaged fruits were harvested. Four ripening stages according to peel colour were used for making the chips: 1 = all green; 2 = green with a little yellow; 3 = green with some yellow; and 4 = yellow with some green. The fruits were left to ripen naturally in a well-aired room to reach each stage. At each stage, fingers were picked randomly from the different bunches, cleaned, peeled manually and thinly sliced into 2-mm-thick slices before frying.

### Frying process

A commercial electric, 6-L capacity, deep fryer (model FR-18-Silver, Fry King, Thailand) was used. In this study, newly refined palm oil (CEMAC par S.C.R. MAYA & ICE, Cameroon) was heated to 170 °C for 10 min to imitate normal frying conditions and kept at a temperature of  $170 \pm 5$  °C. About 500 g of sliced plantain was fried in 3 L of heated oil. Each batch was fried at the set temperature for 5 min. Frying temperature and time used in this study were optimised earlier based on final moisture content and sensory evaluation in comparison with commercial products. After frying, the plantain chips were air-cooled for 5 min then sealed in polyethylene bags and kept at  $25 \pm 2$  °C less than 3 days prior further physico-chemical analysis. The experiment was performed in triplicate.

### Physico-chemical analyses

#### *Analysis of the proximate composition*

Plantain chip moisture content was determined by the AOAC method (1990). The sample was dried in a hot air oven (model UF55; Memmert Oven, Buechenbach, Germany) for 16 h at 105 °C. The moisture content was taken as the weight loss. The Kjeldahl method was used to investigate protein content. Conversion from total nitrogen to percentage crude protein was by a factor of 6.25. The method of AOAC (1990) was used to determine ash content by exposing moisture and all organic constituents to a temperature of 600 °C in a VULCAN<sup>TM</sup> furnace (model 3-1750; Cole-Parmer, IL, USA). The ash content was taken as the residual weight after incineration. The samples' fat content was also calculated by the AOAC method (1990), using the Soxhlet extraction technique (model

FOSS Soxtec<sup>TM</sup> extraction, Sweden). Crude fibre content was established using fibre extraction equipment (model FOSS Fibertec<sup>TM</sup> 2010, Sweden). The carbohydrate content was determined by subtracting the percentages of moisture, crude protein, ash, fat and crude fibre from 100. Atwater's conversion factors were used to calculate the caloric value (kcal per 100 g) according to the caloric coefficients corresponding to the protein (4 kcal g<sup>-1</sup>), carbohydrate (4 kcal g<sup>-1</sup>) and fat (9 kcal g<sup>-1</sup>) contents. All measurements were taken three times.

#### *Determining reducing sugar*

For the sugar analysis, 10 mL of deionised water was added to 1 g of homogenised fresh pulp, then stirred for 10 min. The suspension was centrifuged at 1200 g for 10 min. The supernatant was filtered through a 0.22- $\mu$ m syringe before analysis. High-performance liquid chromatography (HPLC, Agilent Technologies, Cheshire, UK) was used to analyse the amounts of glucose and fructose. The equipment consists of a pump (model LC-20AD; Shimadzu, Kyoto, Japan), a column oven (model CTO-10ASVP; Shimadzu, Kyoto, Japan), a system controller (model CBM-20A; Shimadzu, Kyoto, Japan) and a refractive index detector (model RDI-10A; Shimadzu, Kyoto, Japan). The sugars were separated at a temperature of 80 °C using a carbohydrate column Rezex RNM-column (Phenomenex, Torrance, CA, USA). Deionised water was used to facilitate a flow rate of 0.4 mL min<sup>-1</sup>. Standard calibration curves of sugar standards were used to quantitatively measure each peak as a reference. Results were expressed as g per kg per FW of a sample.

#### *Determining amino acids*

To analyse the amino acids in fresh plantain, the precolumn derivatisation with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) and HPLC with a fluorescent detection procedure was used. The following chemical reagents were purchased: acetonitrile (HPLC super gradient grade) and methanol (HPLC super gradient grade) from Lab-Scan (Dublin, Ireland), hydrochloric acid (36.5%) and trichloroacetic acid from Penta (Chrudim, Czech Republic) and  $\alpha$ -aminobutyric acid from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany). The Milli-Q Plus system (Millipore Corporation, Danvers, MA, USA) was used to produce ultrapure water, while the AccQ-Tag Reagent Kit was bought from Waters (Milford, CT, USA). The reagent kit consists of Waters AccQ-fluor borate buffer, powder (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate-AQC), diluent, amino acid analysing column (Nova-Pak C18, 4  $\mu$ L, 150  $\times$  3.9 mm) and amino acid hydrolysate standard. Each ampoule contains a 2.5 mm mixture of the seventeen hydrolysate amino acids (except for cystine-1.25 mm), including aspartic

acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu) and phenylalanine (Phe).

Five micrograms of the pulverised sample was hydrolysed with 5 mL of constant boiling 6 M HCL in a 10-mL hydrolysis tube using a microwave discover workstation (CEM Microwave Technology Ltd., Buckingham, UK). Each hydrolysate was centrifuged at 1200 g for 10 min to obtain a clear solution. Subsequently, 10  $\mu$ L of the diluted hydrolysate was pipetted into a 6  $\times$  10 mm sample tube, and an equal amount of 0.1 M NaOH was added to neutralise the excess acid before derivatisation. From this, 10  $\mu$ L of the solution was taken for the derivatisation procedure.

In the derivatisation process, 10  $\mu$ L of the diluted hydrolysate and 70  $\mu$ L of AccQ-Fluor Borate Buffer were mixed in a sample tube. The mixture was briefly homogenised with vortex; then, 20  $\mu$ L of reconstituted AccQ-Fluor Reagent was added and mixed again for 60 s. The contents were transferred to an auto-sampler vial that was put in the heating block at 55 °C for 10 min.

A Waters Alliance 2695 HPLC system with a 2475 multi- $\lambda$  fluorescence detector (Waters) was used for the HPLC analysis with an excitation at 250 nm, emission at 395 nm and AccQ-Tag amino acid column Nova-Pak C18 (4  $\mu$ m, 150  $\times$  3.9 mm) (Waters). The column was set at 37 °C with an injection volume of 10  $\mu$ L. A gradient mobile-phase comprised eluent A (prepared from Waters AccQ-Tag Eluent A concentrate, by adding 200 mL of concentrate to 2 L of Milli-Q water and mixing), eluent B (acetonitrile, HPLC grade) and eluent C (Milli-Q water). The best programme for gradient separation was performed at the following intervals: 0 min at 100% A, 0.5 min at 99% A and 1% B, 18 min at 95% A and 5% B, 19 min at 91% A and 9% B, 29.5 min at 83% A and 17% B, 33 min at 60% B and 40% C, 36 min at 100% A and 53 min at 100% A.

To prepare the internal standard, a calibration standard solution was mixed with an internal standard (6.45 mg  $\alpha$ -aminobutyric acid to 25 mL 0.1 M HCl). Then, 40  $\mu$ L of amino acid hydrolysate, an internal standard solution of 40 and 920  $\mu$ L of Milli-Q water were transferred to a sample tube as a stock standard solution. To derivatise the calibration standard, 10  $\mu$ L of the stock standard solution was transferred into a 6  $\times$  10 mm sample tube; 70  $\mu$ L of AccQ-Fluor Borate Buffer was added and vortexed. Thereafter, 20  $\mu$ L of reconstituted AccQ-Fluor Reagent was added, and the solution was mixed for 60 s. The content was then transferred to the bottom of a low volume insert vial and placed on a preheated heating block at 55 °C for 10 min. The process was allowed to stand for few

minutes and 5  $\mu$ L of the derivatised standard was injected into the chromatographic system. Calibration curves were observed to be linear ( $r^2 > 0.990$ ), the limit of detection (LOD) was in the range of 0.01–0.08 mg mL<sup>-1</sup>, and the recovery was between 92% and 108%.

#### Total phenolic analysis

For the first extraction, approximately 1 g of fresh ground samples was mixed with 10 mL of 70% methanol for 10 s before sonication for 10 min. The sample was filtered through Whatman No. 4 paper to obtain a clear solution. In the second extraction, 10 mL of 70% methanol was added over the residue, homogenised on a vortex and dispensed in a water bath at 80 °C for 5 min. The homogenised sample was filtered into a volumetric flask, and extracting solution was added up to a final volume of 25 mL. The extracted sample was then stored at –20 °C before analysis.

The TP content in plantain samples was evaluated using the Folin–Ciocalteu assay as described by Shamla & Nisha (2017) with a slight modification. A total of 400  $\mu$ L of the extracted sample was mixed with 8 mL of distilled water and 0.5 mL of 2 N Folin–Ciocalteu reagent in a test tube. The mixture was shaken continuously and allowed to react for 6 min. Afterwards, the sample was mixed with 1.5 mL of 20% (w v<sup>-1</sup>) sodium carbonate solution then incubated in a water bath at 40 °C for 30 min. The UV-VIS spectrophotometer measured the TP content at 765 nm. Gallic acid was used to plot the standard calibration curve. The TP content was expressed as mg of gallic acid equivalent (GAE) per g of sample.

#### Analysis of carotenoid

Plantain samples were extracted according to a modified procedure described by Amah *et al.* (2019). In short, 5 g of each homogenised sample was weighed and thoroughly mixed with 3 g of celite in 50 mL of cold acetone. The solution was filtered through a filter paper No. 4 in a Büchner funnel. The filtrate was added to 20 mL of petroleum ether in a separating funnel and shaken well by adding 300 mL of distilled water. Subsequently, the lower aqueous-acetone phase was discarded with distilled water and the upper organic phase was collected. The process was repeated 3–4 times for the entire filtrate. The carotenoids in petroleum ether were obtained as light yellow colour extract, and it was passed over funnel containing 25 g anhydrous sodium sulphate to remove traces of distilled water prior the final volume was adjusted to 50 mL with petroleum ether. About 25 mL of the extracted sample was concentrated and dried under nitrogen gas before reconstituting in 1 mL of dichloromethane: methanol (v v<sup>-1</sup>). The solution was then filtered through a 0.22-mm polytetrafluoroethylene

(PTFE) syringe filter (Millipore) into 2-mL vials (Waters PTFE/silicone septum).

The analysis of the extract was carried out on a HPLC system (model Waters Alliance e2695, Waters Corporation) equipped with a photodiode array detector (PDA) and a polymeric YMCTM C30 5 µm column (4.6 × 250 mm). The mobile phase was acetonitrile: ethyl acetate: methanol at the ratio 80:10:10, and carotenoids were detected at wavelength of 450 nm. Sample injection volume was 20 µL and the flow rate was set at 1 mL min<sup>-1</sup> at a temperature of 25 °C. HPLC analysis was done with acetonitrile at pump B and equal mixture of ethyl acetate and methanol at pump A in the ratio 40:60. An external standard method was used to identify lutein, α-carotene, *trans*-β-carotene (*trans*-BC), 13-*cis*-β-carotene (13-*cis*-BC) and 9-*cis*-β-carotene (9-*cis*-BC). Calibration curves were observed to be linear ( $r^2 > 0.999$ ), the LOD was in the range of 0.005–0.020 µg mL<sup>-1</sup>, and the recovery was between 90 and 96%. Total carotenoids (TC) with provitamin A activity were computed as pVACs (µg g<sup>-1</sup> FW) = α-carotene + 13-*cis*-BC + 9-*cis*-BC + *trans*-BC; TC were computed as TC (µg g<sup>-1</sup> FW) = total pVACs + lutein. Provitamin A content expressed in terms of β-carotene equivalents (BCEs) was calculated as BCE (µg g<sup>-1</sup> FW) = 0.5 *trans*-α-carotene + *trans*-BC + 0.53*cis*-BC, where *cis*-BC is the sum of 13-*cis*-BC and 13-*cis*-AC.

#### Determination of acrylamide

The sample preparation for acrylamide analysis was done following the slightly modified method of Shin *et al.* (2010). Two grams of fried plantain chips was put in a 50-mL polypropylene tube; then, 2 mL of [<sup>13</sup>C<sub>3</sub>]-acrylamide (internal standard, 1000 ng mL<sup>-1</sup>) and 18 mL of distilled water were added. The sample was sonicated in an ultrasonic bath for 60 min prior centrifuged at 1500 g for 30 min, and then 2 mL of the supernatant was transferred into a polypropylene tube. A C18 solid-phase extraction cartridge (Sep-Pak Plus, Waters) was activated with 5 mL of methanol and 5 mL of water, respectively; afterwards, the supernatant was applied. To collect all acrylamide in the sample, the residue in the cartridge was eluted again with 2 mL of distilled water. Four millilitres of bromination reagent was added to the collected solution and it was allowed to stand overnight at 4 °C in a refrigerator. The solution was titrated with 1 M of sodium thiosulphate until colourless solution was observed. Then, the solution was mixed with 4 mL of ethyl acetate, shaken for 3 min and centrifuged at 1500 g for 10 min. One millilitre of the supernatant was taken to mix with 100 µL of triethylamine, shaken for 15 min and centrifuged at 3500 g for 5 min. Lastly, 1 mL of the sample solution was compiled for acrylamide analysis using GC–MS method.

The extracts and standards were introduced into a GC–MS system equipment (Agilent Technologies, Palo Alto, CA, USA) equipped with a DB-WAXETR capillary column (30 m × 0.25 mm i.d. 0.25 µm film thickness) (J&W Scientific, Albany, NY, USA). The acrylamide was detected in a mass selective detector operated in selected ion monitoring mode with positive electron impact ionisation. The column was maintained at a temperature of 50 °C for 1 min before increasing to 240 °C and then to 300 °C. The temperature of the injector was set at 250 °C in the splitless mode. The purge time was 1 min, and the transfer line temperature to the mass selective detector was controlled at 280 °C. Acrylamide was separated using helium gas a carrier at a flow rate of 1 mL min<sup>-1</sup>. For quantification, the transitions of *m/z* 149–151 for specific ions of 2-bromopropenamide were used. An isotopically marked [<sup>13</sup>C<sub>3</sub>]-acrylamide with the transition of *m/z* 152–154 for 2-bromo[<sup>13</sup>C<sub>3</sub>]-propenamide was used as an internal standard. A calibration graph was performed by plotting the peak area of acrylamide against the corresponding ratios of the amounts of analyte. Calibration curves were found to be linear ( $r^2 > 0.995$ ), the LOD was 0.07 µg kg<sup>-1</sup>, and the recovery was between 99% and 105%.

#### Data analysis

The effect of different cultivars and ripening stages on the physico-chemical properties of plantain chips was subjected to statistical analyses using the general linear model programme (GLM). Fisher's least significant difference (LSD) estimated the differences among the means of each treatment at 5% of the probability level using the SAS program (version 9.4, SAS, 2002). This test has the lowest critical values acceptable which increases the power to detect an effect or mean difference between groups. The correlations between precursors (reducing sugars, amino acids, TP and carotenoid content) in all the four stages of ripening were studied using Pearson's correlation coefficient.

## Results and discussion

### Proximate composition as affected by cultivars and ripening stages

The results gave a range of 56.8–63.3 g water 100 g<sup>-1</sup> for moisture content at stage 1 for all raw plantain; the difference between the cultivars was not statistically significant (Table 1). The moisture content increased by approximately 4.3% when the fruits were ripened from stage 1 to stage 4. Shamlal & Nisha (2017) reported that during the breakdown of starch into sugars over the ripening time, moisture is released from the fruit peel to the pulp due to osmotic pressure.

**Table 1** Proximate composition and reducing sugars of raw plantains at different ripening stages

Cultivar	Ripening stage	Moisture (g 100 g <sup>-1</sup> )	Crude fat (g 100 g <sup>-1</sup> )	Crude protein (g 100 g <sup>-1</sup> )	Carbohydrate (g 100 g <sup>-1</sup> )	Total ash (g 100 g <sup>-1</sup> )	Crude fibre (g 100 g <sup>-1</sup> )	Gross energy (kcal 100 g <sup>-1</sup> )	Glucose (g 100 g <sup>-1</sup> )	Fructose (g 100 g <sup>-1</sup> )
Local Elat (French bunch type)	1	57.5 (0.4) <sup>ab</sup>	0.3 (0.1) <sup>bd</sup>	2.5 (0.1) <sup>ba</sup>	34.7 (0.4) <sup>ab</sup>	2.0 (0.0) <sup>ab</sup>	3.0 (0.1) <sup>ba</sup>	151.2 (1.3) <sup>ab</sup>	1.6 (0.2) <sup>dc</sup>	6.4 (0.4) <sup>db</sup>
	2	58.4 (0.5) <sup>ac</sup>	0.3 (0.2) <sup>abb</sup>	2.5 (0.0) <sup>ba</sup>	33.9 (0.3) <sup>ab</sup>	2.1 (0.1) <sup>bb</sup>	2.8 (0.0) <sup>bab</sup>	148.6 (1.1) <sup>ab</sup>	2.0 (0.1) <sup>de</sup>	7.3 (0.4) <sup>cc</sup>
	3	59.0 (0.3) <sup>ab</sup>	0.4 (0.0) <sup>abb</sup>	3.1 (0.1) <sup>aa</sup>	32.8 (0.6) <sup>ab</sup>	2.2 (0.0) <sup>ad</sup>	2.6 (0.1) <sup>cb</sup>	146.7 (2.1) <sup>aa</sup>	3.1 (0.0) <sup>be</sup>	8.6 (0.1) <sup>bd</sup>
	4	60.1 (0.4) <sup>ac</sup>	0.5 (0.0) <sup>acb</sup>	3.2 (0.0) <sup>ab</sup>	31.5 (0.5) <sup>ab</sup>	2.3 (0.0) <sup>cc</sup>	2.4 (0.1) <sup>bb</sup>	143.0 (1.5) <sup>aa</sup>	6.7 (0.3) <sup>ad</sup>	11.2 (0.2) <sup>ac</sup>
Batard (False Horn bunch type)	1	56.8 (0.5) <sup>ab</sup>	0.5 (0.3) <sup>bab</sup>	1.6 (0.1) <sup>bd</sup>	36.3 (0.2) <sup>aa</sup>	2.0 (0.0) <sup>cb</sup>	2.9 (0.0) <sup>ba</sup>	155.8 (0.8) <sup>aa</sup>	4.3 (0.0) <sup>da</sup>	4.5 (0.0) <sup>dc</sup>
	2	57.5 (0.2) <sup>abc</sup>	0.5 (0.2) <sup>aba</sup>	1.7 (0.1) <sup>bcd</sup>	35.4 (0.4) <sup>aa</sup>	2.1 (0.0) <sup>bb</sup>	2.8 (0.0) <sup>ba</sup>	152.9 (1.2) <sup>aa</sup>	5.8 (0.1) <sup>ca</sup>	9.3 (1.0) <sup>cb</sup>
	3	58.1 (0.0) <sup>ab</sup>	0.5 (0.2) <sup>aba</sup>	1.8 (0.0) <sup>bc</sup>	34.8 (0.3) <sup>aa</sup>	2.3 (0.1) <sup>ac</sup>	2.4 (0.0) <sup>cc</sup>	151.2 (1.0) <sup>aa</sup>	6.5 (0.2) <sup>ba</sup>	12.0 (0.4) <sup>bb</sup>
	4	58.8 (0.2) <sup>ac</sup>	0.6 (0.1) <sup>aa</sup>	2.2 (0.1) <sup>ac</sup>	33.7 (0.3) <sup>aa</sup>	2.4 (0.3) <sup>abc</sup>	2.4 (0.0) <sup>ab</sup>	148.9 (0.9) <sup>aa</sup>	8.3 (0.1) <sup>ab</sup>	19.5 (0.0) <sup>aa</sup>
Daru (ABB cooking banana)	1	60.5 (0.2) <sup>abc</sup>	0.4 (0.3) <sup>ba</sup>	1.8 (0.1) <sup>cc</sup>	32.5 (0.2) <sup>abc</sup>	2.3 (0.1) <sup>bab</sup>	2.4 (0.0) <sup>ab</sup>	142.0 (2.2) <sup>abc</sup>	4.1 (0.1) <sup>da</sup>	10.6 (0.3) <sup>ca</sup>
	2	60.9 (0.3) <sup>abc</sup>	0.5 (0.6) <sup>aba</sup>	1.9 (0.0) <sup>cbc</sup>	31.8 (0.4) <sup>abc</sup>	2.5 (0.0) <sup>ba</sup>	2.4 (0.1) <sup>abc</sup>	139.5 (1.8) <sup>abc</sup>	5.2 (0.1) <sup>cb</sup>	11.4 (0.1) <sup>ca</sup>
	3	61.5 (0.3) <sup>abc</sup>	0.5 (0.1) <sup>aa</sup>	2.5 (0.0) <sup>bb</sup>	30.5 (0.5) <sup>ab</sup>	2.6 (0.1) <sup>bab</sup>	2.4 (0.1) <sup>abc</sup>	136.7 (1.5) <sup>ab</sup>	6.6 (0.1) <sup>ba</sup>	15.2 (0.1) <sup>ba</sup>
	4	62.1 (0.1) <sup>abc</sup>	0.6 (0.0) <sup>aab</sup>	2.9 (0.1) <sup>aa</sup>	29.5 (0.3) <sup>abc</sup>	2.7 (0.0) <sup>ba</sup>	2.3 (0.0) <sup>bcb</sup>	134.5 (1.3) <sup>ab</sup>	8.8 (0.5) <sup>aa</sup>	18.3 (0.1) <sup>ab</sup>
Hybrid PITA 14 (French bunch type)	1	62.2 (0.5) <sup>aa</sup>	0.4 (0.0) <sup>bcb</sup>	2.0 (0.0) <sup>bb</sup>	30.6 (0.2) <sup>abc</sup>	2.4 (0.1) <sup>bab</sup>	2.4 (0.0) <sup>ab</sup>	134.1 (1.1) <sup>abc</sup>	1.0 (0.1) <sup>dd</sup>	3.4 (0.2) <sup>cd</sup>
	2	63.1 (0.5) <sup>ab</sup>	0.5 (0.2) <sup>aba</sup>	2.1 (0.1) <sup>bb</sup>	29.5 (0.3) <sup>abc</sup>	2.5 (0.0) <sup>bab</sup>	2.4 (0.1) <sup>abc</sup>	130.3 (2.7) <sup>abc</sup>	2.1 (0.1) <sup>de</sup>	4.8 (0.2) <sup>cd</sup>
	3	64.0 (0.2) <sup>ab</sup>	0.5 (0.0) <sup>aa</sup>	2.5 (0.1) <sup>ab</sup>	28.2 (0.1) <sup>ab</sup>	2.5 (0.1) <sup>abb</sup>	2.3 (0.0) <sup>abc</sup>	127.2 (1.0) <sup>ab</sup>	3.6 (0.1) <sup>bd</sup>	5.3 (0.2) <sup>be</sup>
	4	67.1 (0.3) <sup>aa</sup>	0.5 (0.1) <sup>abc</sup>	2.6 (0.1) <sup>ab</sup>	24.9 (0.1) <sup>ad</sup>	2.6 (0.0) <sup>ab</sup>	2.3 (0.0) <sup>bcb</sup>	114.9 (0.7) <sup>ac</sup>	5.0 (0.1) <sup>af</sup>	6.5 (0.1) <sup>ae</sup>
PITA 21 (French bunch type)	1	62.0 (0.4) <sup>aa</sup>	0.3 (0.1) <sup>bd</sup>	1.1 (0.0) <sup>be</sup>	31.7 (0.2) <sup>abc</sup>	2.6 (0.0) <sup>ba</sup>	2.3 (0.1) <sup>ab</sup>	133.7 (0.5) <sup>abc</sup>	1.1 (0.1) <sup>dd</sup>	3.3 (0.1) <sup>dd</sup>
	2	62.6 (0.2) <sup>abc</sup>	0.3 (0.0) <sup>abb</sup>	1.4 (0.1) <sup>abd</sup>	30.8 (0.3) <sup>abc</sup>	2.7 (0.1) <sup>ba</sup>	2.3 (0.0) <sup>cc</sup>	131.5 (0.8) <sup>abc</sup>	3.2 (0.0) <sup>cc</sup>	4.4 (0.1) <sup>cd</sup>
	3	63.3 (0.7) <sup>ab</sup>	0.3 (0.1) <sup>abb</sup>	1.8 (0.1) <sup>ac</sup>	29.7 (0.3) <sup>ab</sup>	2.7 (0.1) <sup>ba</sup>	2.2 (0.1) <sup>bd</sup>	128.7 (0.9) <sup>ab</sup>	5.6 (0.1) <sup>bb</sup>	9.6 (0.0) <sup>bc</sup>
	4	65.5 (0.7) <sup>ab</sup>	0.4 (0.1) <sup>ad</sup>	1.9 (0.0) <sup>ac</sup>	27.3 (0.7) <sup>abcd</sup>	2.7 (0.1) <sup>ba</sup>	2.2 (0.1) <sup>ac</sup>	120.1 (1.6) <sup>abc</sup>	7.6 (0.1) <sup>ac</sup>	11.5 (0.2) <sup>ac</sup>
PITA 27 (French bunch type)	1	63.3 (0.1) <sup>aa</sup>	0.3 (0.0) <sup>bcd</sup>	1.8 (0.1) <sup>dc</sup>	29.1 (0.5) <sup>ac</sup>	2.4 (0.1) <sup>cab</sup>	3.1 (0.0) <sup>aa</sup>	126.4 (1.4) <sup>ac</sup>	1.8 (0.1) <sup>db</sup>	3.8 (0.2) <sup>dd</sup>
	2	63.8 (0.2) <sup>aa</sup>	0.3 (0.1) <sup>bb</sup>	2.0 (0.1) <sup>cb</sup>	28.3 (0.2) <sup>ac</sup>	2.5 (0.0) <sup>ba</sup>	3.1 (0.0) <sup>ba</sup>	124.0 (1.1) <sup>ac</sup>	2.6 (0.0) <sup>cd</sup>	4.5 (0.4) <sup>cd</sup>
	3	64.8 (0.2) <sup>aa</sup>	0.4 (0.1) <sup>abb</sup>	2.5 (0.0) <sup>bb</sup>	26.6 (0.4) <sup>ab</sup>	2.7 (0.1) <sup>ba</sup>	3.0 (0.0) <sup>aba</sup>	119.7 (0.8) <sup>ab</sup>	4.9 (0.2) <sup>bc</sup>	5.2 (0.0) <sup>be</sup>
	4	65.0 (0.2) <sup>ab</sup>	0.4 (0.0) <sup>acd</sup>	2.6 (0.0) <sup>ab</sup>	26.2 (0.5) <sup>acd</sup>	2.8 (0.1) <sup>ba</sup>	2.9 (0.1) <sup>ba</sup>	119.2 (1.3) <sup>abc</sup>	5.3 (0.0) <sup>ae</sup>	7.5 (0.0) <sup>ad</sup>

Maturity stage: 1 = mature green; 2 = green with a trace of yellow; 3 = more green than yellow; and 4 = more yellow than green. Means (± SD) within a column followed by different lower-case letters (ripening stages) and different upper-case letters (cultivars) differ by LSD test ( $P < 0.05$ ).

The increase from 0.3–0.5 to 0.4–0.6 g 100 g<sup>-1</sup> in crude fat was significant ( $P < 0.05$ ), while crude protein rose from 1.1–2.5 to 1.9–3.2 g 100 g<sup>-1</sup> and total ash from 2.0–2.6 to 2.3–2.8 g 100 g<sup>-1</sup> during the process of ripening from stage 1 to stage 4. The level of crude fat and crude protein contents was highly increased in the French plantain ‘Elat’ (40.4%) and PITA 21 (40.9%), respectively, compared with the corresponding values of other cultivars. The breakdown of tissue during ripening may have caused an increase in total ash in ripe plantain probably associated with mineral elements being released. (Baiyeri *et al.*, 2011). The reduction of crude fibre from 2.3–3.1 to 2.2–2.9 g 100 g<sup>-1</sup>, carbohydrate from 29.1–36.3 to 24.9–33.7 g 100 g<sup>-1</sup> and gross energy from 126.4–155.8 to 114.9–148.9 kcal 100 g<sup>-1</sup> was found over the storage time of all cultivars. The carbohydrate content was highest in the False Horn plantain ‘Batard’ at stages 1 and 4, while the least was in hybrid PITA 27 at stage 1 and PITA 21 at stage 4. The reduction of carbohydrates could be explained by the conversion of starch to sugar during the ripening process (Shamla & Nisha, 2017). Eggleston *et al.* (1992) explained the variations in the physico-chemical properties of plantain, hybrids and cooking banana to bunch type and/or environment. They also reported that there was a little less amylose content in hybrids than in plantain and cooking banana.

#### Reducing sugars as affected by cultivars and ripening stages

At stage 1, the initial glucose and fructose contents in all plantain cultivars were in the range of 1.0–4.3 g 100 g<sup>-1</sup> and 3.3–10.6 g 100 g<sup>-1</sup>, respectively (Table 1). The level of glucose and fructose contents significantly varied ( $P < 0.05$ ) by the cultivars. As expected, glucose and fructose contents showed an increase by 48.7–85.7% and 42.1–76.9% from ripening stages 1 to 4, statistically significant at  $P < 0.05$ . Specifically, a higher level of glucose accumulation during the ripening process was observed in the cooking banana ‘Daru’, while the False Horn ‘Batard’ contained the highest level of fructose. Differences in fructose content among all PITA hybrids at stages 1 and 2 were not significant. However, PITA 21 presented the highest level ( $P < 0.05$ ) of both glucose and fructose at stage 4. This agrees with Eggleston *et al.* (1992). They found significantly higher starch and sugar content in the cooking banana than the plantain and their hybrids. Besides, they observed that the starch content of two unripe plantain hybrids was slightly lower than their plantain parent. An increase of glucose and fructose during ripening was because of the degradation of stored starch in the pulp to sugars led to increased glucose and fructose during ripening via various enzymes

including starch phosphorylase and amylase (Mohan *et al.*, 2014). A study by Bhuiyan *et al.* (2020) noted that  $\beta$ -amylase is important in starch breakdown in plantain as the increase in amount of  $\beta$ -amylase at later ripening stage found. In addition, invertase, which is also responsible for starch breakdown, increases at postharvest ripening of plantain according to Iyare & Ekwukoma (1992).

#### Amino acids as affected by cultivars and ripening stages

In this study, it could be seen that all amino acids significantly varied ( $P < 0.05$ ) by cultivars (Table 2). Asparagine, an amino acid indicated as a major precursor to acrylamide formation, ranged from 0.7 to 2.8 mg g<sup>-1</sup> at stage 1. The cooking banana ‘Daru’ contained the highest level of asparagine, while the lowest was found in PITA 14. During the postharvest ripening process, the level of asparagine declined by 9.1–76.5%, depending on the cultivars. The essential amino acid threonine ranged from 0.7 to 1.6 mg g<sup>-1</sup>, valine from 0.2 to 2.1 and phenylalanine from 0.1 to 1.8, displaying a significant reduction ( $P < 0.05$ ) when ripening progressed. On the contrary, the amino acids histidine, methionine, lysine, iso-leucine and leucine, which were in the range of 0.0–0.7, 0.0–0.1, 0.1–0.2 and 0.1–0.7 mg g<sup>-1</sup> at stage 1, significantly increased ( $P < 0.05$ ) by an average of 74.7%, 94.4%, 53.3%, 77.8% and 82.3%, respectively, when the fruits were ripened at stage 4. However, no significant differences were observed in the amino acids serine, glutamine, glycine, arginine, proline, cystine and tyrosine content in all cultivars at postharvest ripening. This agrees with Khawas *et al.* (2014), who reported a decline of essential amino acid content in culinary banana (Musa ABB) during the ripening process. In addition, Shamla & Nisha (2017) also indicated an increase of amino acids serine, iso-leucine, leucine and phenylalanine at stage 4.

#### Total phenolic content as affected by cultivars and ripening stages

The different cultivars had diverse amounts of TP compounds. At stage 1, the average content of TPs varied significantly ( $P < 0.05$ ) from 1.1 to 4.1 mg GAE g<sup>-1</sup> FW (Table 3). A considerably higher concentration was observed in PITA 21, while PITA 14 contained the lowest levels in comparison with the other cultivars at stage 1. However, PITA 14 exhibited the highest content of TPs at stage 4, while the cooking banana ‘Daru’ had the lowest content. As the fruit ripened with more yellow than green, TPs of all cultivars significantly decreased ( $P < 0.05$ ) by approximately 59.4%. The highest degradation of TPs during ripening was found in hybrid PITA 27 (71.9%). This

Table 2 Amino acids (mg g<sup>-1</sup>) of raw plantains at different ripening stages

Cultivar	Ripening stage	Amino acids (mg g <sup>-1</sup> )										
		Asparagine	Serine	Glutamine	Glycine	Histidine	Threonine	Arginine	Alanine	Proline		
Local Elat (French bunch type)	1	1.73 (0.07) <sup>a,c</sup>	0.89 (0.07) <sup>b,c</sup>	0.69 (0.28) <sup>abc</sup>	0.24 (0.14) <sup>bc</sup>	0.24 (0.20) <sup>db</sup>	1.59 (0.00) <sup>aa</sup>	0.94 (0.04) <sup>3c</sup>	2.97 (0.35) <sup>aab</sup>	0.71 (0.00) <sup>bbc</sup>		
	2	1.12 (0.01) <sup>bc</sup>	0.18 (0.00) <sup>bc</sup>	0.60 (0.21) <sup>bc</sup>	0.18 (0.07) <sup>cc</sup>	0.42 (0.00) <sup>cb</sup>	0.62 (0.00) <sup>bc</sup>	0.75 (0.00) <sup>bc</sup>	2.41 (0.00) <sup>bb</sup>	0.29 (0.00) <sup>cc</sup>		
	3	0.69 (0.01) <sup>cb</sup>	0.17 (0.01) <sup>cab</sup>	0.15 (0.00) <sup>de</sup>	0.32 (0.00) <sup>bc</sup>	0.57 (0.00) <sup>bb</sup>	0.16 (0.00) <sup>cd</sup>	0.35 (0.00) <sup>cb</sup>	2.17 (0.30) <sup>cb</sup>	0.37 (0.04) <sup>cd</sup>		
	4	0.41 (0.16) <sup>dd</sup>	1.17 (0.10) <sup>aa</sup>	0.31 (0.11) <sup>cd</sup>	0.24 (0.00) <sup>ac</sup>	0.86 (0.03) <sup>ad</sup>	0.05 (0.07) <sup>dc</sup>	0.36 (0.05) <sup>cc</sup>	2.04 (0.01) <sup>cb</sup>	0.96 (0.14) <sup>ab</sup>		
Batard (False Horn bunch type)	1	1.99 (0.32) <sup>ab</sup>	0.61 (0.11) <sup>ab</sup>	0.15 (0.05) <sup>de</sup>	0.12 (0.05) <sup>ad</sup>	0.12 (0.09) <sup>cc</sup>	1.40 (0.14) <sup>ab</sup>	1.77 (0.00) <sup>aa</sup>	2.91 (0.16) <sup>ab</sup>	0.68 (0.10) <sup>3c</sup>		
	2	1.24 (0.00) <sup>bb</sup>	0.15 (0.00) <sup>bcd</sup>	0.54 (0.00) <sup>bd</sup>	0.08 (0.00) <sup>bd</sup>	0.76 (0.01) <sup>ba</sup>	0.89 (0.06) <sup>bb</sup>	1.12 (0.00) <sup>cb</sup>	2.34 (0.00) <sup>bb</sup>	0.39 (0.00) <sup>de</sup>		
	3	0.85 (0.03) <sup>ca</sup>	0.17 (0.01) <sup>bab</sup>	0.74 (0.02) <sup>ab</sup>	0.02 (0.00) <sup>ce</sup>	0.87 (0.01) <sup>ba</sup>	0.61 (0.00) <sup>cb</sup>	1.23 (0.02) <sup>bca</sup>	2.02 (0.04) <sup>cc</sup>	0.20 (0.05) <sup>de</sup>		
	4	0.68 (0.00) <sup>dab</sup>	0.10 (0.00) <sup>cd</sup>	0.76 (0.00) <sup>ba</sup>	0.06 (0.01) <sup>bd</sup>	1.23 (0.13) <sup>aa</sup>	0.03 (0.01) <sup>dd</sup>	1.26 (0.14) <sup>bb</sup>	1.85 (0.18) <sup>3c</sup>	0.97 (0.13) <sup>ab</sup>		
Daru (ABB cooking banana)	1	2.78 (0.00) <sup>aa</sup>	1.00 (0.00) <sup>aa</sup>	0.45 (0.00) <sup>cd</sup>	1.51 (0.00) <sup>aa</sup>	0.72 (0.00) <sup>da</sup>	1.64 (0.00) <sup>aa</sup>	1.55 (0.00) <sup>bb</sup>	2.98 (0.00) <sup>aa</sup>	1.09 (0.00) <sup>da</sup>		
	2	1.92 (0.28) <sup>ba</sup>	0.43 (0.02) <sup>ba</sup>	0.56 (0.12) <sup>bcd</sup>	1.13 (0.09) <sup>ba</sup>	0.78 (0.06) <sup>ca</sup>	1.13 (0.14) <sup>ba</sup>	1.59 (0.03) <sup>ba</sup>	2.78 (0.05) <sup>ba</sup>	1.61 (0.05) <sup>aa</sup>		
	3	0.85 (0.02) <sup>ca</sup>	0.20 (0.00) <sup>dab</sup>	0.30 (0.01) <sup>dd</sup>	1.28 (0.00) <sup>ba</sup>	0.85 (0.00) <sup>ba</sup>	0.93 (0.00) <sup>ca</sup>	1.25 (0.00) <sup>ca</sup>	2.60 (0.05) <sup>ca</sup>	1.21 (0.01) <sup>ca</sup>		
	4	0.70 (0.00) <sup>da</sup>	0.32 (0.00) <sup>cb</sup>	0.66 (0.00) <sup>ab</sup>	1.55 (0.20) <sup>aa</sup>	1.05 (0.18) <sup>3c</sup>	0.23 (0.01) <sup>da</sup>	1.95 (0.08) <sup>3a</sup>	2.43 (0.07) <sup>da</sup>	1.40 (0.06) <sup>ba</sup>		
Hybrid PITA 14 (French bunch type)	1	0.68 (0.00) <sup>ae</sup>	0.08 (0.03) <sup>abf</sup>	0.65 (0.01) <sup>3c</sup>	0.01 (0.00) <sup>be</sup>	0.03 (0.01) <sup>dd</sup>	0.68 (0.00) <sup>ae</sup>	0.02 (0.01) <sup>3e</sup>	0.36 (0.06) <sup>ad</sup>	0.18 (0.01) <sup>3e</sup>		
	2	0.60 (0.02) <sup>bf</sup>	0.06 (0.01) <sup>be</sup>	0.47 (0.04) <sup>3e</sup>	0.06 (0.02) <sup>ad</sup>	0.14 (0.01) <sup>cd</sup>	0.25 (0.02) <sup>3e</sup>	0.09 (0.00) <sup>3d</sup>	0.32 (0.05) <sup>3d</sup>	0.36 (0.06) <sup>ab</sup>		
	3	0.55 (0.01) <sup>bcd</sup>	0.10 (0.00) <sup>abc</sup>	0.52 (0.02) <sup>3c</sup>	0.05 (0.01) <sup>ae</sup>	0.22 (0.00) <sup>3c</sup>	0.18 (0.02) <sup>3d</sup>	0.07 (0.01) <sup>3bc</sup>	0.28 (0.06) <sup>3de</sup>	0.25 (0.07) <sup>3de</sup>		
	4	0.52 (0.00) <sup>3c</sup>	0.07 (0.00) <sup>3d</sup>	0.55 (0.01) <sup>3c</sup>	0.06 (0.00) <sup>3d</sup>	0.28 (0.01) <sup>3e</sup>	0.02 (0.00) <sup>3e</sup>	0.04 (0.01) <sup>3cd</sup>	0.25 (0.00) <sup>3d</sup>	0.32 (0.00) <sup>3abc</sup>		
PITA 21 (French bunch type)	1	1.20 (0.02) <sup>ad</sup>	0.16 (0.02) <sup>abd</sup>	0.78 (0.01) <sup>3a</sup>	0.65 (0.00) <sup>3b</sup>	0.03 (0.02) <sup>3d</sup>	1.03 (0.12) <sup>3c</sup>	1.13 (0.01) <sup>3a</sup>	0.68 (0.13) <sup>3c</sup>	0.79 (0.02) <sup>3b</sup>		
	2	0.88 (0.00) <sup>3d</sup>	0.12 (0.00) <sup>3d</sup>	0.68 (0.00) <sup>3d</sup>	0.51 (0.00) <sup>3b</sup>	0.19 (0.00) <sup>3c</sup>	1.13 (0.00) <sup>3a</sup>	0.03 (0.00) <sup>3de</sup>	0.43 (0.01) <sup>3c</sup>	0.25 (0.05) <sup>3c</sup>		
	3	0.70 (0.00) <sup>3b</sup>	0.15 (0.04) <sup>3abc</sup>	0.97 (0.03) <sup>3a</sup>	0.70 (0.12) <sup>3a</sup>	0.57 (0.06) <sup>3b</sup>	0.32 (0.03) <sup>3c</sup>	0.15 (0.15) <sup>3e</sup>	0.40 (0.04) <sup>3d</sup>	0.93 (0.08) <sup>3ab</sup>		
	4	0.54 (0.22) <sup>3c</sup>	0.18 (0.00) <sup>3c</sup>	0.75 (0.00) <sup>3a</sup>	0.55 (0.01) <sup>3b</sup>	1.12 (0.01) <sup>3ab</sup>	0.11 (0.01) <sup>3b</sup>	0.08 (0.00) <sup>3d</sup>	0.27 (0.02) <sup>3d</sup>	0.22 (0.03) <sup>3d</sup>		
PITA 27 (French bunch type)	1	0.72 (0.00) <sup>ae</sup>	0.12 (0.01) <sup>3e</sup>	0.72 (0.02) <sup>3b</sup>	0.12 (0.01) <sup>3d</sup>	0.11 (0.01) <sup>3c</sup>	0.92 (0.01) <sup>3d</sup>	0.02 (0.00) <sup>3e</sup>	0.39 (0.02) <sup>3d</sup>	0.49 (0.01) <sup>3d</sup>		
	2	0.69 (0.01) <sup>3de</sup>	0.23 (0.07) <sup>3b</sup>	0.76 (0.01) <sup>3a</sup>	0.17 (0.07) <sup>3ab</sup>	0.13 (0.03) <sup>3d</sup>	0.36 (0.01) <sup>3d</sup>	0.01 (0.01) <sup>3e</sup>	0.31 (0.00) <sup>3d</sup>	0.27 (0.00) <sup>3dc</sup>		
	3	0.66 (0.02) <sup>3bc</sup>	0.22 (0.03) <sup>3ba</sup>	0.75 (0.00) <sup>3bb</sup>	0.19 (0.06) <sup>3d</sup>	0.30 (0.00) <sup>3e</sup>	0.10 (0.00) <sup>3d</sup>	0.08 (0.02) <sup>3c</sup>	0.25 (0.00) <sup>3e</sup>	0.78 (0.05) <sup>3c</sup>		
	4	0.65 (0.05) <sup>3bb</sup>	0.18 (0.00) <sup>3c</sup>	0.74 (0.00) <sup>3bc</sup>	0.12 (0.00) <sup>3d</sup>	0.32 (0.01) <sup>3e</sup>	0.03 (0.01) <sup>3d</sup>	0.05 (0.00) <sup>3d</sup>	0.20 (0.03) <sup>3d</sup>	0.39 (0.01) <sup>3c</sup>		
Local Elat (French bunch type)	1	0.07 (0.00) <sup>3c</sup>	0.32 (0.00) <sup>3b</sup>	0.81 (0.01) <sup>3c</sup>	0.81 (0.01) <sup>3c</sup>	0.14 (0.00) <sup>3d</sup>	0.60 (0.00) <sup>3d</sup>	0.12 (0.06) <sup>3ca</sup>	0.12 (0.04) <sup>3bb</sup>	1.51 (0.07) <sup>3ab</sup>		
	2	0.06 (0.00) <sup>3c</sup>	0.11 (0.00) <sup>3b</sup>	0.46 (0.01) <sup>3b</sup>	0.46 (0.01) <sup>3b</sup>	0.34 (0.01) <sup>3ca</sup>	1.22 (0.01) <sup>3ca</sup>	0.25 (0.01) <sup>3ca</sup>	0.14 (0.01) <sup>3bb</sup>	0.57 (0.07) <sup>3bb</sup>		
	3	0.04 (0.00) <sup>3c</sup>	0.33 (0.02) <sup>3b</sup>	0.26 (0.00) <sup>3ca</sup>	1.70 (0.08) <sup>3ba</sup>	1.47 (0.05) <sup>3ba</sup>	1.47 (0.05) <sup>3ba</sup>	1.58 (0.00) <sup>3ba</sup>	0.23 (0.06) <sup>3bb</sup>	0.31 (0.00) <sup>3cb</sup>		
	4	0.06 (0.01) <sup>3c</sup>	0.25 (0.03) <sup>3ab</sup>	0.11 (0.00) <sup>3dab</sup>	2.54 (0.06) <sup>3a</sup>	1.81 (0.22) <sup>3a</sup>	1.81 (0.22) <sup>3a</sup>	2.05 (0.22) <sup>3a</sup>	1.25 (0.05) <sup>3ba</sup>	0.21 (0.02) <sup>3ca</sup>		
Batard (False Horn bunch type)	1	0.07 (0.02) <sup>3bc</sup>	0.15 (0.11) <sup>3c</sup>	1.65 (0.19) <sup>3ab</sup>	0.01 (0.00) <sup>3d</sup>	0.49 (0.10) <sup>3ab</sup>	0.51 (0.00) <sup>3ab</sup>	0.13 (0.11) <sup>3da</sup>	0.13 (0.08) <sup>3bb</sup>	0.54 (0.01) <sup>3d</sup>		
	2	0.17 (0.01) <sup>3ab</sup>	0.14 (0.00) <sup>3b</sup>	0.54 (0.00) <sup>3b</sup>	0.25 (0.00) <sup>3b</sup>	0.25 (0.00) <sup>3b</sup>	0.48 (0.01) <sup>3b</sup>	0.24 (0.00) <sup>3ca</sup>	0.18 (0.01) <sup>3cb</sup>	0.42 (0.01) <sup>3c</sup>		
	3	0.06 (0.00) <sup>3c</sup>	0.09 (0.03) <sup>3c</sup>	0.28 (0.09) <sup>3ca</sup>	0.28 (0.09) <sup>3ca</sup>	0.48 (0.01) <sup>3b</sup>	0.59 (0.00) <sup>3b</sup>	0.64 (0.01) <sup>3c</sup>	0.24 (0.00) <sup>3bb</sup>	0.23 (0.05) <sup>3bc</sup>		
	4	0.10 (0.02) <sup>3abb</sup>	0.10 (0.03) <sup>3c</sup>	0.17 (0.01) <sup>3ca</sup>	1.73 (0.06) <sup>3ab</sup>	0.63 (0.06) <sup>3c</sup>	1.06 (0.01) <sup>3c</sup>	1.06 (0.01) <sup>3c</sup>	0.82 (0.01) <sup>3c</sup>	0.14 (0.01) <sup>3bab</sup>		
Daru (ABB cooking banana)	1	0.68 (0.00) <sup>3ca</sup>	1.73 (0.00) <sup>3a</sup>	2.05 (0.00) <sup>3a</sup>	0.03 (0.00) <sup>3d</sup>	0.47 (0.00) <sup>3d</sup>	0.13 (0.00) <sup>3da</sup>	0.07 (0.00) <sup>3d</sup>	0.07 (0.00) <sup>3d</sup>	1.40 (0.00) <sup>3c</sup>		
	2	0.56 (0.00) <sup>3da</sup>	1.22 (0.10) <sup>3ba</sup>	1.43 (0.04) <sup>3ba</sup>	0.35 (0.00) <sup>3ca</sup>	0.46 (0.02) <sup>3b</sup>	0.24 (0.09) <sup>3ca</sup>	0.09 (0.01) <sup>3ab</sup>	0.09 (0.01) <sup>3ab</sup>	1.20 (0.09) <sup>3ba</sup>		
	3	0.76 (0.04) <sup>3ba</sup>	1.31 (0.07) <sup>3ba</sup>	0.35 (0.02) <sup>3ca</sup>	0.49 (0.07) <sup>3b</sup>	0.56 (0.07) <sup>3b</sup>	0.86 (0.00) <sup>3bb</sup>	0.12 (0.01) <sup>3abc</sup>	0.12 (0.01) <sup>3abc</sup>	0.23 (0.01) <sup>3c</sup>		
	4	0.99 (0.08) <sup>3ba</sup>	1.38 (0.17) <sup>3ba</sup>	0.16 (0.01) <sup>3da</sup>	1.17 (0.13) <sup>3ac</sup>	0.85 (0.01) <sup>3ab</sup>	1.79 (0.01) <sup>3ab</sup>	0.16 (0.01) <sup>3ab</sup>	0.16 (0.01) <sup>3ab</sup>	0.12 (0.01) <sup>3db</sup>		
Hybrid PITA 14 (French bunch type)	1	0.06 (0.00) <sup>3c</sup>	0.13 (0.02) <sup>3cd</sup>	0.17 (0.00) <sup>3e</sup>	0.01 (0.01) <sup>3c</sup>	0.07 (0.03) <sup>3de</sup>	0.07 (0.03) <sup>3de</sup>	0.07 (0.03) <sup>3ba</sup>	0.09 (0.01) <sup>3c</sup>	0.09 (0.01) <sup>3ef</sup>		
	2	0.06 (0.00) <sup>3c</sup>	0.08 (0.01) <sup>3b</sup>	0.10 (0.09) <sup>3bd</sup>	0.13 (0.00) <sup>3bc</sup>	0.17 (0.01) <sup>3c</sup>	0.09 (0.02) <sup>3bb</sup>	0.32 (0.00) <sup>3ba</sup>	0.05 (0.00) <sup>3bd</sup>	0.05 (0.00) <sup>3bd</sup>		



Table 2 (Continued)

Cultivar	Ripening stage	Cystine	Tyrosine	Valine	Methionine	Lysine	Iso-leucine	Leucine	Phenylalanine
PITA 21 (French bunch type)	3	0.04 (0.00) <sup>bc</sup>	0.11 (0.00) <sup>abc</sup>	0.07 (0.02) <sup>bcb</sup>	0.26 (0.02) <sup>abc</sup>	0.31 (0.01) <sup>bc</sup>	0.15 (0.00) <sup>de</sup>	0.51 (0.00) <sup>ba</sup>	0.03 (0.01) <sup>de</sup>
	4	0.07 (0.01) <sup>abc</sup>	0.12 (0.01) <sup>ac</sup>	0.05 (0.00) <sup>cb</sup>	0.35 (0.02) <sup>de</sup>	0.57 (0.02) <sup>ac</sup>	0.19 (0.01) <sup>de</sup>	1.09 (0.03) <sup>ab</sup>	0.02 (0.00) <sup>dc</sup>
	1	0.07 (0.01) <sup>ac</sup>	0.16 (0.05) <sup>abc</sup>	0.41 (0.02) <sup>ad</sup>	0.01 (0.01) <sup>cd</sup>	0.37 (0.13) <sup>cc</sup>	0.10 (0.00) <sup>ca</sup>	0.05 (0.01) <sup>cd</sup>	1.83 (0.69) <sup>ba</sup>
	2	0.06 (0.00) <sup>ac</sup>	0.19 (0.00) <sup>ab</sup>	0.27 (0.00) <sup>bc</sup>	0.09 (0.00) <sup>cd</sup>	0.46 (0.00) <sup>bb</sup>	0.13 (0.00) <sup>cb</sup>	0.13 (0.00) <sup>cb</sup>	1.15 (0.07) <sup>ba</sup>
PITA 27 (French bunch type)	3	0.06 (0.00) <sup>ac</sup>	0.06 (0.03) <sup>cc</sup>	0.13 (0.04) <sup>cb</sup>	0.49 (0.11) <sup>bb</sup>	0.49 (0.02) <sup>bb</sup>	0.26 (0.00) <sup>bd</sup>	0.26 (0.05) <sup>bb</sup>	0.67 (0.01) <sup>ca</sup>
	4	0.08 (0.03) <sup>abc</sup>	0.12 (0.01) <sup>bc</sup>	0.08 (0.00) <sup>cab</sup>	0.53 (0.00) <sup>ad</sup>	0.58 (0.00) <sup>ac</sup>	0.65 (0.01) <sup>ad</sup>	0.65 (0.01) <sup>ad</sup>	0.21 (0.01) <sup>da</sup>
	1	0.15 (0.01) <sup>ab</sup>	0.09 (0.00) <sup>ad</sup>	0.17 (0.05) <sup>ae</sup>	0.01 (0.00) <sup>bc</sup>	0.45 (0.01) <sup>bbc</sup>	0.15 (0.01) <sup>ca</sup>	0.02 (0.01) <sup>de</sup>	0.39 (0.01) <sup>de</sup>
	2	0.06 (0.00) <sup>bc</sup>	0.11 (0.00) <sup>ab</sup>	0.15 (0.01) <sup>ad</sup>	0.02 (0.01) <sup>be</sup>	0.46 (0.07) <sup>abb</sup>	0.14 (0.01) <sup>bcb</sup>	0.12 (0.03) <sup>bb</sup>	0.11 (0.00) <sup>bd</sup>
	3	0.11 (0.02) <sup>abb</sup>	0.13 (0.07) <sup>ac</sup>	0.13 (0.01) <sup>ab</sup>	0.05 (0.01) <sup>ad</sup>	0.49 (0.10) <sup>abb</sup>	0.17 (0.01) <sup>abe</sup>	0.24 (0.00) <sup>bb</sup>	0.08 (0.00) <sup>cd</sup>
	4	0.06 (0.00) <sup>bc</sup>	0.11 (0.00) <sup>ac</sup>	0.10 (0.02) <sup>aab</sup>	0.06 (0.02) <sup>af</sup>	0.62 (0.01) <sup>ac</sup>	0.21 (0.00) <sup>de</sup>	0.66 (0.01) <sup>ad</sup>	0.01 (0.00) <sup>dc</sup>

Maturity stage: 1 = mature green; 2 = green with a trace of yellow; 3 = more green than yellow; and 4 = more yellow than green. Means (± SD) within a column followed by different lower-case letters (ripening stages) and different upper-case letters (cultivars) differ by LSD test ( $P < 0.05$ ).

results like that of Shamla & Nicha (2017), who noted a decline in TP and total flavonoid contents in plantain during ripening. Parr & Bolwell (2000) explained a reduction in TP to an increase of polyphenol oxidase (PPO) activity and an increased polymerisation of leucoanthocyanidins during fruit ripening as well as to losing astringency via hydrolysis of astringent arabinose esters of hexahydrodiphenic acid. Borges *et al.* (2019) also indicated that the bioactive amines, particularly serotonin and dopamine, which also act as antioxidants, decreased until stage 5 (mostly yellow) and increased at stage 7. Reduced serotonin and dopamine levels in banana cultivars are associated with oxidation activated during ripening (Borges *et al.*, 2019). While Ben-Ahmed *et al.* (2009) ascribed a reduction in TP content during ripening to the fact that various phenolic compounds can form complex compounds, such as tannin and lignin, that could not be investigated by the analytical method used.

**Carotenoid content as affected by cultivars and ripening stages**

All analysed plantain cultivars showed a significant variation ( $P < 0.05$ ) in carotenoid content (Table 3). It could be seen that the average concentration of lutein,  $\alpha$ -carotene, 13-*cis*-BC, 9-*cis*-BC and *trans*-BC at stage 1 varied widely from 0.0 to 0.6, 0.2 to 4.9, 0.4 to 4.1, 0.2 to 0.3 and 0.1 to 0.3  $\mu\text{g g}^{-1}$  FW, respectively. At this stage, the French plantain ‘Elat’ showed the highest value of pVACs, TC and BCE among the cultivars, followed by Batard and Daru. When the ripening progressed, lutein,  $\alpha$ -carotene, 13-*cis*-BC, 9-*cis*-BC and *trans*-BC concentration significantly increased ( $P < 0.05$ ) in all cultivars by an average of 75.1%, 78.1%, 86.5%, 38.7% and 64.1%, respectively. At stage 4, the highest value of pVACs, TC and BCE was found in the False Horn ‘Batard’, followed by Elat and Daru, respectively. It could be also seen that the carotenoid content of all hybrids with a French-type bunch was significantly lower ( $P < 0.05$ ) than the French ‘Elat’. Similarly, Amah *et al.* (2019) reported the highest proportion of pVACs (88%) in plantain compared to *Musa acuminata* cultivars (78%) and hybrids (67%).

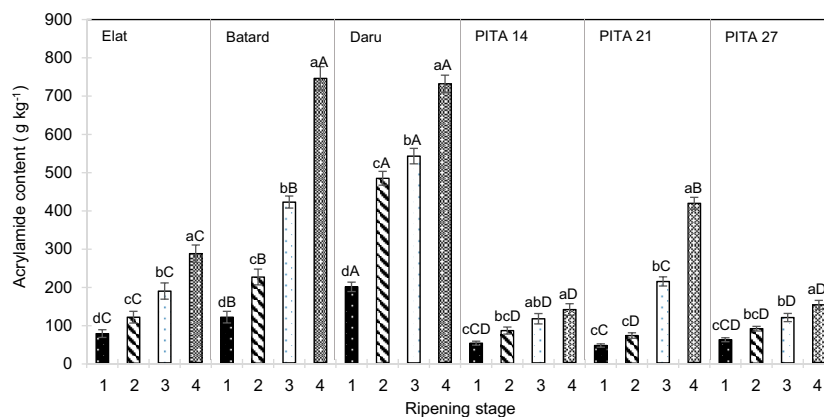
Alos *et al.* (2019) ascribed increased carotenoids in ripe pulp to degraded chlorophylls accompanied by a rise of carotenoids. Moreover, the different levels of carotenoid accumulation during ripening in each cultivar could be due to the different expressions and regulation of carotenogenesis gene transcripts and the structure and function of diverse metabolic enzymes (Ma *et al.*, 2018) caused by the various growing conditions at the different sampling sites. Udomkun *et al.* (2020) indicated that at each ripening stage pVAC concentration differed across locations. Also, they

Table 3 Total phenolic and carotenoid contents of raw plantains at different ripening stages

Cultivar	Ripening stage	TPC (mg GAE g <sup>-1</sup> )	Lutein (µg g <sup>-1</sup> FW)	α-carotene (µg g <sup>-1</sup> FW)	13-cis-BC (µg g <sup>-1</sup> FW)	9-cis-BC (µg g <sup>-1</sup> FW)	trans-BC (µg g <sup>-1</sup> FW)	pVACs (µg g <sup>-1</sup> FW)	TC (µg g <sup>-1</sup> FW)	BCE (µg g <sup>-1</sup> FW)
Local Elat (French bunch type)	1	1.61 (0.05) <sup>ad</sup>	0.38 (0.15) <sup>bcb</sup>	4.85 (0.84) <sup>ia</sup>	4.05 (0.66) <sup>ca</sup>	0.31 (0.05) <sup>ia</sup>	0.26 (0.12) <sup>ba</sup>	9.48 (0.25) <sup>ia</sup>	9.86 (0.03) <sup>ba</sup>	4.83 (0.86) <sup>ba</sup>
	2	1.30 (0.06) <sup>bd</sup>	0.84 (0.04) <sup>aa</sup>	5.09 (0.92) <sup>ca</sup>	4.22 (0.46) <sup>ca</sup>	0.51 (0.06) <sup>ca</sup>	0.43 (0.13) <sup>ba</sup>	10.25 (0.10) <sup>ca</sup>	11.08 (0.00) <sup>ba</sup>	5.21 (0.81) <sup>ba</sup>
	3	1.11 (0.04) <sup>bc</sup>	0.93 (0.02) <sup>acd</sup>	6.52 (0.33) <sup>ba</sup>	5.59 (0.39) <sup>ba</sup>	0.66 (0.09) <sup>ba</sup>	0.60 (0.02) <sup>ba</sup>	13.37 (0.17) <sup>ba</sup>	14.30 (0.01) <sup>ba</sup>	6.82 (0.96) <sup>aba</sup>
	4	0.64 (0.02) <sup>db</sup>	0.98 (0.01) <sup>ae</sup>	8.85 (0.06) <sup>ab</sup>	7.32 (0.04) <sup>ab</sup>	1.22 (0.04) <sup>ab</sup>	1.41 (0.02) <sup>ab</sup>	18.79 (0.13) <sup>ab</sup>	19.78 (0.03) <sup>ab</sup>	9.71 (0.10) <sup>ab</sup>
Batard (False Horn bunch type)	1	1.13 (0.02) <sup>ae</sup>	0.27 (0.01) <sup>ccd</sup>	0.70 (0.03) <sup>bc</sup>	1.93 (0.07) <sup>db</sup>	0.25 (0.01) <sup>cab</sup>	0.19 (0.01) <sup>cb</sup>	3.08 (0.10) <sup>db</sup>	3.34 (0.01) <sup>db</sup>	1.57 (0.05) <sup>db</sup>
	2	0.97 (0.04) <sup>be</sup>	0.52 (0.00) <sup>bc</sup>	1.42 (0.31) <sup>cd</sup>	3.72 (0.42) <sup>cb</sup>	0.50 (0.00) <sup>ba</sup>	0.42 (0.04) <sup>ba</sup>	6.06 (0.62) <sup>cb</sup>	6.57 (0.01) <sup>cb</sup>	3.10 (0.39) <sup>cb</sup>
	3	0.81 (0.05) <sup>cd</sup>	0.55 (0.00) <sup>be</sup>	5.19 (0.00) <sup>bb</sup>	5.80 (0.00) <sup>ba</sup>	0.57 (0.00) <sup>ba</sup>	0.55 (0.00) <sup>ba</sup>	12.11 (0.00) <sup>bb</sup>	12.66 (0.01) <sup>bb</sup>	6.22 (0.00) <sup>bb</sup>
	4	0.65 (0.01) <sup>db</sup>	0.74 (0.02) <sup>af</sup>	11.27 (0.13) <sup>ba</sup>	7.54 (0.04) <sup>ab</sup>	0.94 (0.01) <sup>ac</sup>	0.65 (0.00) <sup>ab</sup>	20.39 (0.11) <sup>ba</sup>	21.13 (0.00) <sup>ba</sup>	10.28 (0.09) <sup>ba</sup>
Daru (ABB cooking banana)	1	0.99 (0.06) <sup>af</sup>	0.02 (0.00) <sup>de</sup>	0.21 (0.00) <sup>de</sup>	0.38 (0.00) <sup>de</sup>	0.17 (0.00) <sup>db</sup>	0.07 (0.00) <sup>bd</sup>	0.82 (0.00) <sup>bd</sup>	0.84 (0.00) <sup>de</sup>	0.37 (0.00) <sup>de</sup>
	2	0.75 (0.06) <sup>bf</sup>	0.36 (0.03) <sup>cd</sup>	1.09 (0.22) <sup>de</sup>	2.04 (0.93) <sup>cc</sup>	0.23 (0.02) <sup>bb</sup>	0.20 (0.01) <sup>cbc</sup>	3.55 (0.51) <sup>cc</sup>	3.91 (0.01) <sup>cd</sup>	1.82 (0.05) <sup>cd</sup>
	3	0.57 (0.03) <sup>ca</sup>	0.99 (0.03) <sup>bc</sup>	1.30 (0.12) <sup>bf</sup>	5.30 (0.15) <sup>bb</sup>	0.44 (0.02) <sup>bb</sup>	0.32 (0.02) <sup>bb</sup>	7.36 (0.25) <sup>bd</sup>	8.35 (0.00) <sup>bd</sup>	3.78 (0.16) <sup>bd</sup>
	4	0.41 (0.03) <sup>cd</sup>	1.19 (0.02) <sup>ad</sup>	1.86 (0.02) <sup>be</sup>	9.04 (0.09) <sup>aa</sup>	1.13 (0.01) <sup>ab</sup>	0.45 (0.02) <sup>bc</sup>	12.48 (0.08) <sup>bc</sup>	13.67 (0.01) <sup>bc</sup>	6.17 (0.04) <sup>ac</sup>
Hybrid PITA 14 (French bunch type)	1	3.10 (0.03) <sup>ab</sup>	0.21 (0.00) <sup>dd</sup>	0.90 (0.13) <sup>bb</sup>	0.95 (0.09) <sup>bc</sup>	0.19 (0.00) <sup>cab</sup>	0.08 (0.00) <sup>bcd</sup>	2.11 (0.16) <sup>dc</sup>	2.32 (0.01) <sup>dc</sup>	1.03 (0.11) <sup>dc</sup>
	2	2.85 (0.01) <sup>bb</sup>	0.71 (0.06) <sup>cb</sup>	1.84 (0.14) <sup>bc</sup>	1.25 (0.47) <sup>bd</sup>	0.22 (0.01) <sup>cb</sup>	0.29 (0.01) <sup>bb</sup>	3.59 (0.86) <sup>cd</sup>	4.30 (0.03) <sup>cd</sup>	1.87 (0.62) <sup>cd</sup>
	3	2.12 (0.03) <sup>cb</sup>	1.11 (0.14) <sup>bb</sup>	2.92 (0.18) <sup>be</sup>	2.34 (0.06) <sup>ae</sup>	0.36 (0.07) <sup>bbc</sup>	0.61 (0.04) <sup>ba</sup>	6.24 (0.26) <sup>bf</sup>	7.35 (0.03) <sup>be</sup>	3.31 (0.21) <sup>bf</sup>
	4	1.56 (0.02) <sup>ca</sup>	2.56 (0.00) <sup>aa</sup>	3.81 (0.02) <sup>ad</sup>	2.44 (0.05) <sup>ae</sup>	0.51 (0.01) <sup>ad</sup>	0.76 (0.02) <sup>ab</sup>	7.52 (0.09) <sup>ae</sup>	10.09 (0.01) <sup>ae</sup>	3.96 (0.05) <sup>ae</sup>
PITA 21 (French bunch type)	1	4.12 (0.01) <sup>dab</sup>	0.49 (0.01) <sup>dab</sup>	0.37 (0.05) <sup>bd</sup>	0.39 (0.04) <sup>de</sup>	0.15 (0.00) <sup>cb</sup>	0.07 (0.00) <sup>bd</sup>	0.97 (0.07) <sup>bd</sup>	1.46 (0.00) <sup>bd</sup>	0.46 (0.05) <sup>bd</sup>
	2	3.08 (0.08) <sup>ba</sup>	0.70 (0.00) <sup>cb</sup>	1.69 (0.10) <sup>bc</sup>	1.40 (0.04) <sup>cd</sup>	0.18 (0.00) <sup>bcb</sup>	0.14 (0.00) <sup>bc</sup>	3.41 (0.09) <sup>cd</sup>	4.11 (0.03) <sup>cd</sup>	1.73 (0.07) <sup>cd</sup>
	3	2.73 (0.09) <sup>ca</sup>	0.84 (0.14) <sup>bd</sup>	3.62 (0.18) <sup>bc</sup>	3.91 (0.34) <sup>bc</sup>	0.24 (0.02) <sup>bd</sup>	0.26 (0.01) <sup>ab</sup>	8.02 (0.67) <sup>bc</sup>	8.86 (0.01) <sup>bc</sup>	4.14 (0.49) <sup>bc</sup>
	4	1.52 (0.04) <sup>da</sup>	1.53 (0.02) <sup>ac</sup>	6.64 (0.25) <sup>bc</sup>	4.46 (0.33) <sup>ad</sup>	0.36 (0.08) <sup>be</sup>	0.31 (0.13) <sup>ad</sup>	11.76 (0.67) <sup>ac</sup>	13.29 (0.03) <sup>acd</sup>	5.99 (0.43) <sup>ac</sup>
PITA 27 (French bunch type)	1	2.71 (0.04) <sup>ba</sup>	0.58 (0.02) <sup>ca</sup>	0.85 (0.06) <sup>ab</sup>	0.80 (0.06) <sup>dd</sup>	0.19 (0.01) <sup>bab</sup>	0.11 (0.06) <sup>cc</sup>	1.95 (0.14) <sup>dc</sup>	2.53 (0.03) <sup>dc</sup>	0.96 (0.08) <sup>dc</sup>
	2	1.77 (0.03) <sup>bc</sup>	0.71 (0.01) <sup>cb</sup>	2.22 (0.02) <sup>cb</sup>	2.00 (0.01) <sup>cc</sup>	0.22 (0.00) <sup>abc</sup>	0.19 (0.00) <sup>bcb</sup>	4.63 (0.02) <sup>cc</sup>	5.33 (0.01) <sup>cc</sup>	2.36 (0.02) <sup>cc</sup>
	3	1.12 (0.03) <sup>cc</sup>	1.43 (0.20) <sup>ba</sup>	3.37 (0.09) <sup>bd</sup>	2.99 (0.08) <sup>bd</sup>	0.26 (0.00) <sup>acd</sup>	0.25 (0.00) <sup>abb</sup>	6.87 (0.13) <sup>be</sup>	8.30 (0.01) <sup>bd</sup>	3.52 (0.09) <sup>be</sup>
	4	0.49 (0.07) <sup>dc</sup>	2.29 (0.12) <sup>ab</sup>	3.88 (0.30) <sup>ad</sup>	5.91 (0.31) <sup>ac</sup>	0.31 (0.02) <sup>be</sup>	0.32 (0.01) <sup>ad</sup>	10.41 (0.48) <sup>ad</sup>	12.70 (0.00) <sup>ad</sup>	5.39 (0.33) <sup>ad</sup>

Maturity stage: 1 = mature green; 2 = green with a trace of yellow; 3 = more green than yellow; and 4 = more yellow than green. Means (± SD) within a column followed by different lower-case letters (ripening stages) and different upper-case letters (cultivars), differ by LSD test ( $P < 0.05$ ).

13-cis-BC, 13-cis-β-carotene; 9-cis-BC, 9-cis-β-carotene; BCE, β-carotene equivalents; pVACs, provitamin A carotenoids; TC, total carotenoids; TPC, total phenolic content; trans-BC, trans-β-carotene.



**Figure 1** Acrylamide concentration in plantain chips according to cultivar and ripening stage. Means ( $\pm$ SD) within a bar graph followed by different lower-case letters (ripening stages) and different upper-case letters (cultivars), differ by LSD test ( $P < 0.05$ ).

reported that the interaction between ripening stage, cultivar, bunch type and location significantly contributed to pVACs variability in plantain. On the other hand, the declining level of carotenoids during ripening was noted by Ngoh Newilah *et al.* (2009). This mechanism could be explained by the enzymatic cleavage of carotenoids, which may activate the synthesis of other carotenoid isomers like volatile compounds (Borges *et al.*, 2019).

#### Acrylamide formation as affected by cultivars and ripening stages

Acrylamide concentration in fried plantain chips from stage 1 was in the range of 38.1–190.1  $\mu\text{g kg}^{-1}$ , depending on the cultivar (Fig. 1). Specifically, the cooking banana ‘Daru’ at stage 1 had the highest acrylamide concentration, followed by Batard and Elat, respectively. Among the French bunch types, a significantly higher ( $P < 0.05$ ) acrylamide concentration was found in ‘Elat’, while PITA 21 exhibited the lowest levels. Varying by cultivars, the concentration of acrylamide was significantly increased ( $P < 0.05$ ) during the ripening process. An increase of 44.9% for Elat, 67.3% for Batard, 44.9% for Daru, 23.0% for PITA 14, 77.3% for PITA 21 and 17.4% for PITA 27 was observed when the fruits ripened at stage 4. At this stage, the highest value of acrylamide was identified in Batard, followed by Daru and Elat, respectively. In all hybrids, the level of acrylamide formation in PITA 27 and PITA 14 at stage 4 was not significantly different and was significantly lower ( $P < 0.05$ ) than in Elat and PITA 21. Increased concentration of acrylamide in plantain chips could be caused by different mechanisms such as the Maillard reaction. The formation of this carcinogenic compound involves the interaction between the free amino group of amino acids and the carbonyl group of reducing sugars during the heating process (Friedman, 2015), the decarboxylation and deamination of the amino acid asparagine (Yaylayan

*et al.*, 2005) and/or the presence of phenolic compounds (Zhu *et al.*, 2010).

#### Factors affecting acrylamide formation in plantain chips

To analyse factors influencing acrylamide formation in fried chips, the Pearson correlation ( $r$ ) was performed to determine the correlation between the ripening stage and other chemical precursors (Table 4). It could be seen that the ripening stage significantly influenced ( $P < 0.05$ ) acrylamide formation in deep-fried chips with an  $r$  value of 0.57. The result also showed that the levels of glucose and fructose during plantain ripening significantly and linearly correlated ( $P < 0.05$ ) with acrylamide formation with  $r$  values of 0.85 and 0.96, respectively. Although amino acid asparagine alone can generate acrylamide via a thermal decarboxylation and deamination reaction, reducing sugars are necessary to convert asparagine into acrylamide (Yaylayan *et al.*, 2005). A finding in this study agrees with a report of Rydberg *et al.* (2005), who indicated that fructose plays a vital role in generating acrylamide in heated foods compared to glucose. Nonetheless, Robert *et al.* (2004) reported that the aldehyde group in glucose has more impact on acrylamide production than the ketohexose group in fructose when they studied acrylamide formation from asparagine under low-moisture Maillard reaction conditions.

Considering the effect of amino acids, it could be observed that the level of asparagine, which is reported to be the main precursor of acrylamide formation, showed an insignificant and very weak correlation ( $r = -0.07$ ) with acrylamide formation in plantain chips (Table 4). At the same time, the formation of acrylamide was significantly related ( $P < 0.05$ ) to the level of the amino acids glycine ( $r = 0.48$ ), histidine ( $r = 0.89$ ), arginine ( $r = 0.63$ ), alanine ( $r = 0.42$ ), proline ( $r = 0.59$ ), cystine ( $r = 0.61$ ), tyrosine ( $r = 0.48$ ), methionine ( $r = 0.54$ ) and iso-leucine ( $r = 0.62$ ). There are numerous plausible reaction routes by which

**Table 4** Pearson correlation between cultivars, ripening stages, and chemical factors and acrylamide formation in deep-fried plantain chips

Factors	Pearson correlation ( <i>r</i> )	<i>P</i> -value	<i>R</i> <sup>2</sup>
Ripening stage	0.569	<0.001	0.32
Reducing sugars			
Glucose	0.848	<0.001	0.72
Fructose	0.960	<0.001	0.92
Amino acids			
Asparagine	-0.067	0.651	0.01
Serine	0.015	0.919	0.00
Glutamine	0.005	0.976	0.00
Glycine	0.477	0.001	0.23
Histidine	0.891	<0.001	0.79
Threonine	-0.219	0.135	0.05
Arginine	0.628	<0.001	0.39
Alanine	0.419	0.003	0.18
Proline	0.586	<0.001	0.34
Cystine	0.605	<0.001	0.37
Tyrosine	0.480	0.001	0.23
Valine	-0.014	0.927	0.00
Methionine	0.541	<0.001	0.29
Lysine	0.205	0.161	0.04
Iso-leucine	0.622	<0.001	0.39
Leucine	0.068	0.648	0.00
Phenylalanine	-0.208	0.155	0.04
Total phenolic	-0.616	<0.001	0.38
Carotenoids			
Lutein	0.066	0.657	0.00
$\alpha$ -carotene	0.386	0.007	0.15
<i>trans</i> -BC	0.716	<0.001	0.51
13- <i>cis</i> -BC	0.290	0.045	0.08
9- <i>cis</i> -BC	0.640	<0.001	0.41

amino acids may form acrylamide without going through acrolein. Within the frame of complex, multi-stage reaction mechanisms, involving hydrolyses, rearrangements, decarboxylations, deaminations, etc., have been proposed. However, it is not possible to point out any specific routes, or to exclude any possibilities (Lingnert *et al.*, 2002). Though the amino acid asparagine is one of the strong precursors in generating acrylamide through the decarboxylation and deamination reactions, it is not the case of this study. The possible pathway of this phenomenon might be explained by the generation of acrylamide via the reaction of ammonia with acrylic acid from other amino acids. Yaylayan *et al.* (2005) studied acrylamide formation in model systems. They demonstrated that acrylic acid can be generated directly from certain amino acids or dipeptides such as carnosine,  $\beta$ -alanine, aspartic acid or indirectly from amino acids cysteine and serine. Also, they reported that cysteine and serine were found to reduce pyruvic acid to lactic acid, which can be converted into acrylic acid and form acrylamide compounds when it reacts to ammonia. Furthermore,

Friedman & Levin (2008) reported that the concentration of asparagine does not have a significant effect on acrylamide formation in potatoes, while Yoshida *et al.* (2005) indicated that reducing sugars is the limiting factor for acrylamide formation in potato chips, not the content of asparagine in the tubers.

When the level of TP content is considered, it was observed that a higher level of TP was significantly related ( $P < 0.05$ ) to a lower level of acrylamide ( $r = -0.62$ ) (Table 4). This implies that the reduction of TP content in plantain during ripening was also involved in acrylamide formation in deep-fried chips. Passo Tsamo *et al.* (2015) showed that hydroxycinnamic acids, particularly ferulic acid-hexoside, dominated as the major phenolic compounds in the pulp of the following plantain cultivars: Red Yade, Mbeta 1, Big Ebanga, Moto Ebanga, Batard, Essong, Mbouroukou 1 and Mbouroukou 3. A large diversity was seen among cultivars. In contrast, synaptic acid-hexoside and myricetin-deoxyhexose-hexoside were also present in most plantain cultivars. A study by Shamla & Nisha (2017) found that gallic acid, chlorogenic acid, syringic acid, *p*-coumaric acid and quercetin were identified as major phenolic compounds in the plantain cultivar Nendran. These phenolic acids have been reported as their potential antioxidant in inhibiting/mitigating the formation of carcinogenic/neurotoxic acrylamides by trapping carbonyl during the Maillard reaction (Kalita *et al.*, 2013). Likewise, a positive consequence of phenolic compound chlorogenic acid, which could abstract reactive free electrons from the reactive intermediates formed during the Maillard reaction, was hypothesised by Zhu *et al.* (2010). However, conflicting results have been reported that some phenolic compounds with the higher hydrophilic property might increase the acrylamide formation (Zhu *et al.*, 2009).

Regarding carotenoid content effect on acrylamide formation, it could be highlighted that all carotenoid isomers, except lutein, showed a positive and significant correlation ( $P < 0.05$ ) with acrylamide formation. Specifically, *trans*-BC exhibited the higher correlation with *r* value of 0.72, followed by 9-*cis*-BC ( $r = 0.64$ ),  $\alpha$ -carotene ( $r = 0.39$ ) and 13-*cis*-BC ( $r = 0.29$ ), respectively. This means an increase of these carotenoid isomers during postharvest ripening caused a higher level of acrylamide. Though antioxidant compounds can decrease acrylamide formation, the correlation between acrylamide content and antioxidant activity in some cases is controversial. In this case, the possible mechanism that could explain this variable result is the complexity of the acrylamide pathway as acrylamide is produced in series reactions between the amino acid asparagine and a carbonyl compound producing various intermediates. Therefore, molecular structure and functional groups can affect antioxidant compounds, including carotenoids. This interference can promote

or reduce acrylamide formation despite its antioxidant activity (Kahkeshani *et al.*, 2015). Moreover, Jin *et al.* (2013) mentioned that related parameters influence acrylamide levels, such as system matrix, concentration and type of antioxidants, temperature, heating time, pH and moisture content that can cause opposite results.

## Conclusions

This study provides data on the alteration of the chemical properties of two plantain cultivars (French and False Horn bunch types), three plantain hybrids and a cooking banana during ripening and illustrated the effect of these chemical factors on the acrylamide formation in deep-fried chips of starchy bananas. Plantains at stages 1–3 are usually selected for producing chips. The results showed that the cooking banana ‘Daru’ at these stages exhibited the highest level of acrylamide formation when compared to plantains and plantain hybrids, respectively. At the same ripening stage, the False Horn ‘Batard’ had a higher level of acrylamide than the French ‘Elat’. Comparing French and French hybrids at stages 1–3, a significant lower acrylamide concentration was also found in all plantain hybrids. Likewise, the acrylamide formation in plantain chips showed a significant positive correlation with ripening stages. The limiting factor for acrylamide formation in plantain chips was reducing sugars (glucose and fructose), not amino acid asparagine content. However, the formation of acrylamide was significantly related to other amino acids histidine, arginine, iso-leucine and cystine. It was also observed that a reduction of TP during the ripening process highly increased the level of acrylamide, while carotenoid isomers, except lutein, showed an opposite result. To alleviate the bioavailability of acrylamide in deep-fried chips, therefore, the proper cultivar needs to be selected and the plantain hybrids seem to offer an advantage over the landraces. In addition, plantains with lower and more predictable acrylamide-forming potential should be introduced by breeders and agronomists in the future. Also, the methods to decrease the concentration of acrylamide precursors in plantain while maintaining desirable nutritional and sensory properties should be conducted.

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## Conflict of interest

The authors have no conflict of interest.

## Author contribution

**Patchimaporn Udomkun:** Conceptualization (lead); Formal analysis (lead); Methodology (lead); Writing-original draft (lead). **Rony Swennen:** Investigation (supporting); Supervision (equal); Validation (equal); Writing-review & editing (equal). **Cargele Masso:** Funding acquisition (equal); Investigation (equal); Writing-review & editing (equal). **Bhundit Innawong:** Conceptualization (equal); Methodology (equal); Writing-review & editing (equal). **Apollin Fotso Kuate:** Writing-review & editing (equal). **Amos Alakonya:** Funding acquisition (equal); Writing-review & editing (equal). **Bernard Vanlauwe:** Supervision (equal); Writing-review & editing (equal).

## Ethical approval

This study does not involve any human or animal testing.

## Peer review

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## Data availability statement

This manuscript has no associated data.

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