



## COMPARATIVE PROXIMATE COMPOSITION AND TOTAL PHENOLIC CONTENTS OF FOUR *Musa* CULTIVARS AT UNRIPE AND RIPE STAGES

Oladele, S. O.

Department of Agricultural and Environmental Engineering, Federal University of Technology, Akure, Nigeria

Correspondence: sooladele@futa.edu.ng

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### Abstract

Bananas (*Musa* spp.) are a staple in the Nigerian diet and supply nutrients and recognised bioactives. Yet comparative information linking cultivar genetics, ripening stage and antioxidant retention is limited. This study quantified and compared proximate composition and total phenolic content (TPC) in four cultivars (*M. sapientum*, *M. acuminata* Colla, *M. balbisiana* and *M. × paradisiaca*) harvested at unripe and ripe stages. Moisture content, ash, protein, fat and fibre were determined by AOAC Official Methods, and carbohydrate was calculated by difference; TPC was assessed with the Folin–Ciocalteu assay. Triplicate data ( $n = 3$ ) were analysed by one-way ANOVA with Duncan's test ( $p < 0.05$ ). Unripe samples contained 0.42–0.57 mg gallic-acid equivalents (GAE)  $100\text{ g}^{-1}$  fresh weight (FW), whereas ripe fruit ranged from 4.68 to 10.70 mg GAE  $100\text{ g}^{-1}$  FW. All cultivars were carbohydrate-rich (76–83 %) and very low in fat ( $< 1.5\%$ ). Ripe *M. balbisiana* recorded the highest TPC, 2.3-fold above ripe *M. acuminata* ( $p < 0.05$ ). Antioxidant retention depends on genotype and maturity, with B-genome cultivars preserving more phenolics upon ripening. The low-fat levels endorse their use in heart-healthy diets, while the high phenolic load of ripe fruit supports reported gastro-protective effects. Results guide processors in choosing cultivar and maturity for specific functional products—such as carbohydrate-rich, low-fat flours from unripe pulp and antioxidant-fortified ingredients from ripe *M. balbisiana*—and reduce post-harvest losses.

**Keywords:** Banana flour; total phenolic content, Folin–Ciocalteu assay, ripening physiology, functional foods

### Introduction

Fruits are the sweet or sour, fleshy organs of seed-bearing plants usually eaten raw. Bananas and plantains (*Musa* spp.) are cultivated in more than 150 countries and underpin food and income security across most tropical and subtropical regions. With approximately 139 million t harvested globally in 2023 (FAO, 2023), bananas are the world's most-produced fruit crop and rank fourth among staple foods after rice, wheat and maize. Nigeria is a major African producer, recording 7.3 million t in 2023 (Knoema, 2023; CA, 2025.), chiefly from the humid south but with increasing expansion into the drier savannah under irrigation.

*Musa* comprises more than 1,000 recognised cultivars, which are taxonomically classified based on ploidy levels and the genomic contributions of *Musa acuminata* (A genome) and *Musa balbisiana* (B genome) (Heslop-Harrison and Schwarzacher, 2007; Perrier et al., 2011). Dessert bananas are predominantly A-genome, whereas plantains and cooking bananas possess substantial B-genome or are A × B hybrids (Pillay et al., 2016). Bananas are

nutrient-dense as they supply rapidly available carbohydrates, dietary fibre, potassium and vitamin C. Their phenolic and  $\beta$ -carotene contents underpin reported antihypertensive, antidiabetic and neuroprotective activities (Qusti et al., 2010; Kamyab et al., 2020). Typical pulp total-phenolic concentrations range from 0.15 to 55.5 mg GAE  $\text{g}^{-1}$  fresh weight across diverse cultivar (Bashmil et al., 2021), while functional banana-flour products have recorded TPC values greater than 1 100 mg GAE  $\text{kg}^{-1}$  (Kritsi et al., 2023). These bioactives drive intense interest in banana-derived functional foods.

Bananas are highly perishable; post-harvest losses during transport and marketing can reach 25–50 % in West Africa (Mathias et al., 2024). To curb losses and diversify usage, unripe pulp is often dried and milled into flour for bakery, weaning and gluten-free formulations (Abiodun-Solanke and Falade, 2011; Pragati et al., 2019). Banana fruit contains a diverse range of bioactive compounds, including phenolics, carotenoids, vitamin C, flavonoids, amines and vitamin E, all of which enhance its antioxidant capacity and related health benefits (Pillay et al.,

2016). The principal phenolics identified are catechin, tannins, epicatechin, gallic acid and anthocyanins (Kandasamy and Aradhya, 2014). These phenolic compounds display anti-inflammatory, antibacterial, antiallergenic and antiviral properties, thereby supporting disease prevention and overall well-being.

From the existing research, Shini et al. (2024) evaluated unripe green banana flours from ten Indian cultivars and reported that they possess high levels of resistant starch (~41.8% d/w in Malayannan), along with appreciable concentrations of phenolics, amino acids, and vitamins. Antioxidant capacity, assessed via DPPH and ABTS assays, was notably highest in the Robusta and Palayankodan flours. However, few investigations compare nutrient and antioxidant profiles simultaneously across ripening stages and across genomic groups (A, B, A × B hybrids). Comparative data that link genomic background, maturity stage and bioactive retention remain scarce. Such information is essential for optimising cultivar-specific processing strategies and formulating value-added products tailored to nutritional goals.

The hypothesis of this study is that cultivars with higher B-genome content will retain greater total phenolic and antioxidant capacity in the unripe state than A-genome dessert bananas, owing to reported genotype-dependent differences in secondary-metabolite biosynthesis. This study therefore quantified and compared the proximate composition and antioxidant properties of four representative cultivars, namely *Musa sapientum*, *Musa acuminata* Colla, *Musa balbisiana*, and *Musa* × *paradisiaca*, at both unripe and ripe stages. Outcomes will guide processors in selecting maturity stages and cultivars that maximise functional quality, while contributing to post-harvest loss reduction through value-added utilisation.

## Material and Methods

### Source of Samples

Fresh and physiologically mature fruits of four banana cultivars, namely *Musa sapientum*, *Musa acuminata* Colla, *Musa balbisiana*, and *Musa paradisiaca*, were procured from King's Market (Oja-Oba) in Akure, Ondo State, Nigeria. The fruits were sorted into ripe and unripe groups, after which they were peeled, rinsed with clean water, and sliced uniformly.

### Proximate Composition Determination

The proximate composition of the unripe banana flours was determined to quantify moisture, ash, crude protein, crude fat, and crude fibre contents. All analyses were performed in triplicate using standard procedures described by the Association of Official

Analytical Chemists (AOAC, 2019). Results were reported on a dry weight basis.

Prior to analysis, each flour sample was passed through a 60-mesh sieve to ensure uniform particle size. The sieved samples were then sealed in airtight polyethylene containers and stored at 4 °C to preserve their integrity.

Moisture content was determined following AOAC Method 925.10. A two-gram portion of flour was weighed into a pre-dried crucible and dried in a hot-air oven at 105 °C until constant weight was achieved. The moisture content was calculated from the loss in weight after drying.

Ash content was measured using AOAC Method 923.03. Approximately two grams of each sample were placed in porcelain crucibles and incinerated in a muffle furnace at 550 °C for five hours. The crucibles were cooled in a desiccator and reweighed. The remaining residue was recorded as ash and expressed as a percentage of the initial sample mass.

Crude protein was estimated by the Kjeldahl method (AOAC Method 979.09). One gram of flour was digested with concentrated sulphuric acid in the presence of potassium sulphate and copper sulphate as catalysts. The resulting digest was neutralised with sodium hydroxide and distilled. The liberated ammonia was collected in boric acid solution and titrated with 0.1 normal hydrochloric acid. The nitrogen content obtained was multiplied by the standard conversion factor of 6.25 to derive the crude protein content.

Crude fat was determined by Soxhlet extraction using AOAC Method 920.39. Two grams of flour were extracted with petroleum ether (boiling point 40 to 60 °C) for a period of six hours. The solvent was then evaporated, and the recovered residue was dried and weighed to estimate fat content.

Crude fibre content was determined in accordance with AOAC Method 962.09. Two grams of defatted flour were boiled sequentially in 1.25 percent sulphuric acid and 1.25 percent sodium hydroxide solutions. The residue was filtered, dried, and weighed. It was subsequently ashed at 550 °C, cooled in a desiccator, and reweighed. Fibre content was calculated from the difference in weight before and after ashing.

Carbohydrate (%) was calculated by difference, as shown in Equation 1 (Olabinjo and Adeniyani, 2020; Oparaku et al., 2010).

$$\% \text{Carbohydrate} = 100 - (\% \text{Ash} + \% \text{Protein} + \% \text{Moisture} + \% \text{Crude Fibre} + \% \text{Fat}) \quad (1)$$

### Total Phenolic Content Determination

The total phenolic content of the banana flour extracts was determined using the Folin–Ciocalteu

colorimetric assay as described by Singleton et al. (1999). Results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of dry weight. All samples were analysed in triplicate.

The total phenolic content of the banana flour samples was determined using the Folin–Ciocalteu colorimetric assay, following the procedure outlined by Singleton et al. (1999). One gram of each sample was extracted with 10 mL of 80 percent methanol under continuous shaking at 200 revolutions per minute for two hours at ambient temperature. The mixture was centrifuged at 4,000 revolutions per minute for ten minutes to obtain a clear supernatant, which was used for analysis.

For the assay, 0.5 mL of the extract was transferred into a clean test tube. This was followed by the addition of 2.5 mL of ten percent Folin–Ciocalteu reagent and 2.0 mL of 7.5 percent sodium carbonate solution. The mixture was vortexed and allowed to incubate in the dark at room temperature for thirty minutes. After incubation, the absorbance of the solution was measured at 765 nanometres using a UV–Visible spectrophotometer. A reagent blank, containing methanol instead of the extract, was also prepared to account for background absorbance.

A standard calibration curve was prepared using gallic acid solutions ranging from 0 to 200 micrograms per millilitre. The phenolic content of each sample was extrapolated from the calibration curve and reported as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dw). All determinations were carried out in triplicate to ensure reliability of results.

#### Statistical Analysis

Data ( $n = 3$ ) were processed using IBM SPSS Statistics, version 16.0 (IBM Corp., 2023).

Differences among means were evaluated by one-way ANOVA followed by Duncan's multiple-range test at  $p < 0.05$ .

## Results and Discussion

### Proximate Composition

Table 1 summarises the proximate values for the four Musa cultivars at unripe and ripe stages.

Moisture content in the unripe samples ranged from 9.20 to 12.01 percent. *Musa sapientum* recorded the lowest value, while *M. paradisiaca* had the highest. After ripening, moisture content decreased to a range of 5.09 to 6.56 percent. The order remained consistent, with *M. paradisiaca* retaining the highest value and *M. sapientum* the lowest. As shown in Table 1, moisture content reduced during ripening. This observation contradicts the increase reported by Kookal and Thimmaiah (2018), which may be due to environmental or varietal differences. The approximate 40 percent reduction in moisture content suggests progressive water loss during tissue softening. Ripening is characterised by increased enzymatic activity, which breaks down complex polysaccharides and weakens cell wall structures. This promotes tissue softening and raises cell membrane permeability. As a result, water is lost through transpiration and evaporation. The reduction in moisture content may also result from heightened respiration and solute-driven water efflux from the cells (Wills et al., 2007). In the unripe fruit, ash content ranged from 1.99 % to 4.31 %, with *Musa balbisiana* exhibiting the highest value and *M. acuminata* the lowest. After ripening, ash converged to 2.06–2.07 %; only *M. acuminata* showed a slight increase. These shifts support the view of Khawas et al. (2014) that mineral content varies with the fruit's developmental stage and differential mineral uptake.

**Table 1:** Proximate composition of unripe and ripe *Musa* spp varieties (g/100 g)

Nutrient	<i>M. sapientum</i>		<i>M. acuminata</i> <i>Colla</i>		<i>M. balbisiana</i>		<i>M. × paradisiaca</i>	
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
Moisture	9.20 ± 0.01 <sup>a</sup>	5.09 ± 0.86 <sup>a</sup>	11.07 ± 0.03 <sup>b</sup>	6.01 ± 0.03 <sup>a</sup>	11.34 ± 0.07 <sup>c</sup>	6.04 ± 0.00 <sup>a</sup>	12.01 ± 0.09 <sup>d</sup>	6.56 ± 0.46 <sup>a</sup>
Ash	2.33 ± 0.05 <sup>b</sup>	2.07 ± 0.02 <sup>a</sup>	1.99 ± 0.02 <sup>a</sup>	2.06 ± 0.03 <sup>a</sup>	4.31 ± 0.02 <sup>d</sup>	2.07 ± 0.03 <sup>a</sup>	3.94 ± 0.05 <sup>c</sup>	2.07 ± 0.07 <sup>a</sup>
Fat	0.00	2.21 ± 0.01 <sup>a</sup>	0.00	2.15 ± 0.04 <sup>a</sup>	0.00	2.07 ± 0.00 <sup>a</sup>	0.00	2.07 ± 0.00 <sup>a</sup>
Fibre	3.48 ± 0.03 <sup>b</sup>	0.75 ± 0.00 <sup>d</sup>	4.29 ± 0.02 <sup>c</sup>	0.67 ± 0.00 <sup>c</sup>	3.53 ± 0.00 <sup>b</sup>	0.57 ± 0.01 <sup>b</sup>	3.30 ± 0.02 <sup>a</sup>	0.49 ± 0.04 <sup>a</sup>
Protein	0.10 ± 0.02 <sup>a</sup>	10.30 ± 0.01 <sup>d</sup>	0.13 ± 0.01 <sup>ab</sup>	9.26 ± 0.06 <sup>c</sup>	0.17 ± 0.02 <sup>b</sup>	8.49 ± 0.00 <sup>b</sup>	0.13 ± 0.10 <sup>a</sup>	8.28 ± 0.03 <sup>a</sup>
Carbohydrate	84.90 ± 0.02 <sup>c</sup>	79.60 ± 0.90 <sup>a</sup>	82.52 ± 0.07 <sup>b</sup>	79.85 ± 0.10 <sup>a</sup>	80.66 ± 0.08 <sup>a</sup>	80.77 ± 0.03 <sup>a</sup>	80.62 ± 0.06 <sup>a</sup>	80.39 ± 0.68 <sup>a</sup>

Different superscript letters within a row denote significant differences ( $p < 0.05$ ; Duncan test,  $n = 3$ )

Fat content was undetectable ( $< 0.01\%$ ) in all unripe samples but increased to 2.07–2.22 % on ripening, peaking in *M. × paradisiaca*. The rise indicates modest lipid accumulation during maturation; unripe pulp therefore contributes negligible fat, whereas ripe fruit supplies small amounts of fat-soluble nutrients (Agunloye et al., 2024; Netshiheni et al., 2019). This small but consistent increase accords with reports that lipid biosynthesis accelerates late in maturation.

Unripe cultivars contained 3.30–4.29 % fibre, highest in *M. acuminata* and lowest in plantain. Ripening reduced fibre contents to 0.49–0.75 %, with *M. sapientum* retaining the most. Similar declines have been reported by Egbebi and Bademosi (2012) and Kookal and Thimmaiah (2018) for banana and plantain. Fibre fell 7- to 9-fold after ripening, consistent with cell-wall solubilisation. High-fibre foods aid bowel function and toxin elimination (Obiageli et al., 2016).

Protein increased from 0.10–0.17 % in unripe fruit to 8.28–10.30 % in ripe fruit; *M. sapientum* showed the greatest increase. Comparable maturation-related increases were noted by Kookal and Thimmaiah (2018) and may reflect varietal, climatic or altitudinal effects (Akaninwor and Sodge, 2005). Protein increased markedly on ripening; the sharp rise may reflect conversion from storage forms or concentration effects as moisture declined.

Carbohydrate dominated the proximate profile. Unripe samples contained 80.63–84.90 %, lowest in plantain and highest in *M. sapientum*. After ripening, values fell slightly to 79.60–80.77 %, possibly due to starch-to-sugar conversion and fibre loss. In contrast, Kookal and Thimmaiah (2018) reported a carbohydrate increase during ripening of banana, a discrepancy that may arise from genotype or environmental conditions (Akaninwor and Sodge, 2005). Carbohydrate dominated the proximate

profile ( $> 79\%$  in all ripe samples), confirming bananas and plantain as high-energy foods.

### Total Phenolic Content

The bar chart in Figure 1 presents the total phenolic content (TPC) of unripe and ripe samples across four *Musa* species. The phenolic content is expressed in mg gallic acid equivalent per 100 g dry weight (mg GAE 100 g<sup>-1</sup>). The data demonstrate marked differences in TPC between unripe and ripe stages for all varieties studied.

Across all cultivars, ripening significantly increased the total phenolic content. This aligns with the general biochemical trend whereby phenolic synthesis is upregulated during fruit maturation due to enhanced secondary metabolism and oxidative stress response (Shahidi and Naczki, 2004). Phenolic compounds play a protective role against ripening-induced oxidative degradation, which explains their elevated concentrations in ripe tissues.

*M. acuminata* (B) exhibited the highest phenolic concentration post-ripening, reaching approximately 10.8 mg GAE 100 g<sup>-1</sup>. This suggests a superior antioxidant potential, possibly due to its genetic predisposition to synthesise phenylpropanoids and flavonoids. *M. sapientum* (A) followed with a moderately high value, while *M. balbisiana* (C) and *M. × paradisiaca* (D) presented comparatively lower values.

In the unripe state, all cultivars displayed minimal phenolic levels, ranging from 0.5 to 1.0 mg GAE 100 g<sup>-1</sup>. This is expected, as early fruit development prioritises carbohydrate accumulation and structural cell wall formation over secondary metabolite biosynthesis (Ayala-Zavala et al., 2004).

The statistical annotations (a–d) indicate significant differences ( $p < 0.05$ ) among the cultivars at each

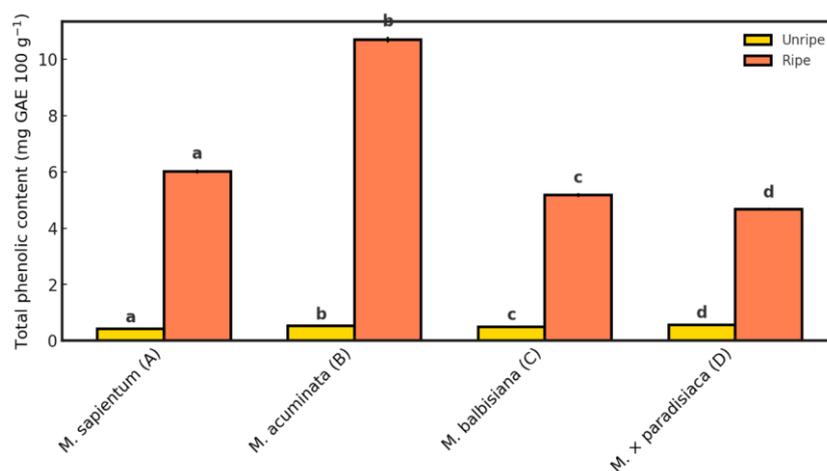


Figure 1: Total phenolic content profile of unripe and ripe *Musa* spp varieties

stage, confirming genotypic variation in phenolic expression. The highest TPC in ripe *M. acuminata* and the lowest in ripe *M. × paradisiaca* emphasise the influence of genetic background on phenolic metabolism.

The rise in TPC with ripening enhances the antioxidant quality of banana flours, which is beneficial for functional food applications. Additionally, the varietal differences in phenolic content suggest that cultivar selection is crucial when targeting health-promoting banana-based products.

### Conclusions

This study confirms that ripening stage strongly shapes the nutrient and phenolic profiles of four *Musa* cultivars. Carbohydrate remained high ( $\approx 80$  % dry weight) and fat consistently low ( $< 2$  %), supporting the use of unripe pulp as a low-fat energy source suitable for cardiovascular-friendly diets. Total phenolic content increased from 0.42–0.57 mg GAE 100 g<sup>-1</sup> in unripe fruit to 4.68–10.70 mg GAE 100 g<sup>-1</sup> after ripening, an eight- to twenty-one-fold rise. The B-dominant AB hybrid *M. × paradisiaca* exhibited the highest unripe phenolics, whereas the pure A-genome dessert banana *M. acuminata* recorded the greatest ripe value, partially supporting the hypothesis that genomic background influences phenolic retention. Absolute TPC values were lower than most published ranges, a divergence that may reflect cultivar genetics, environmental conditions or extraction efficiency and warrants further investigation. These findings provide processors with stage- and genotype-specific data for formulating value-added products and highlight the need for expanded analyses encompassing minerals, antinutrients and individual phenolic compounds.

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