

ANTIOXIDANT ACTIVITY, PHYTOCHEMICAL AND ANTIOXIDANT LEVELS OF *MUSA PARADISIACA* L. AND *MUSA SAPIENTUM* L. AT VARIOUS RIPENING STAGES**Dr. B.O. Agoreyo**

Affiliation: Department Of Biochemistry, Faculty of Life Sciences, University Of Benin, Benin City, Nigeria

Dr. F.O. Agoreyo

Affiliation: Department Of Physiology, School Of Basic Medical Sciences, University Of Benin, Benin City, Nigeria)

Dr. M.I. Omigie

Affiliation: Department Of Physiology, School Of Basic Medical Sciences, University Of Benin, Benin City, Nigeria)

CORRESPONDING AUTHOR: DR. F.O. AGOREYO (DEPARTMENT OF PHYSIOLOGY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, NIGERIA. Phone Number: +2348035628857

ABSTRACT: *Musa paradisiaca* L. (plantain) and *Musa sapientum* L. (banana) are tropical fruits that play a major role in the nutrition and health of people throughout the world. Analyses of the levels of antioxidants such as glutathione, carotenoids and vitamin E of two cultivars of *Musa paradisiaca* and three cultivars of *Musa sapientum* revealed an increase in these antioxidants from the unripe to the overripe stage during ripening. The overripe stages of *Musa paradisiaca* L.cv. French (Bini plantain) and *Musa sapientum* L.cv. *Bluggoe cacambou* (Cooking banana) were found to contain the highest level of glutathione (54.10 ± 0.60 µg/g fresh weight and 47.79 ± 3.45 µg/g fresh weight, respectively). The highest level of lycopene occurred in the overripe stages of *Musa paradisiaca* L. cv. False horn (Auchi plantain) and *Musa sapientum* L.cv. *Bluggoe cacambou* (Cooking banana) with values 0.91 ± 0.00 and 0.80 ± 0.01 µg/g fresh weight, respectively. The highest level of vitamin E (20.20 ± 1.99 µg/g fresh weight and 17.53 ± 1.18 µg/g fresh weight) occurred in *Musa paradisiaca* L.cv False horn (Auchi plantain) and *Musa sapientum* L.cv *Dwarf Cavendish* (English banana). However β-carotene was detected only in the unripe stage of *Musa paradisiaca* L.cv False horn (Auchi plantain) and the level of β-carotene was negligible. Phytochemical screening of the plantain and banana cultivars showed decreased levels of tannins, phenols and alkaloids but increased levels of saponins and flavonoids as ripening progressed except in *Musa sapientum* L.cv. *Bluggoe cacambou* (Cooking banana) where there was a decrease in the level of saponins. Antioxidant activity also increased with ripening in the plantain and banana cultivars, with their ripe and overripe stages having the highest values. Methanolic extracts of the plantain and banana cultivars showed higher antioxidant activity than that of aqueous extracts. The results obtained in this study showed that plantain and banana irrespective of the variety are good sources of antioxidants particularly when they are ripe and overripe.

KEYWORDS: *Musa paradisiaca*, *Musa sapientum*, Antioxidant, Phytochemical, Ripening**INTRODUCTION**

Fruits and vegetables are important components of a healthy diet (Kumar *et al.*, 2012). Fruits are rich sources of antioxidants that help in lowering the incidence of degenerative diseases such as cancer, arthritis, neurodegenerative and cardiovascular diseases (Feskanich *et al.*, 2000). Plantain and Banana belong to the family *Musaceae* and the genus *Musa*. They constitute the fourth most important global food after rice, wheat and maize. They are grown in more than 100 countries over a harvested area of approximately 10 million hectares. Plantains and Bananas although they contain mostly starch but they are classified as fruits (Nelson *et al.*, 2006).

In India plantain is referred to as ‘coarse banana’, in Ghana, plantain is the third most important food crop after yam and cassava in terms of volume of production and contributes 13.1% to the agricultural gross domestic product (Idachaba, 2000 ; Pari, 2000). Plantain is a multi-purpose crop which can be consumed and used at all stages of ripening. It is one of the most important staple food crops consumed by millions of people in developing countries. It reaches its greatest importance in West and Central Africa where more than 10 million tons are produced annually, which are traded locally. In part of East Africa the annual consumption is over 200 kg per capital (International institute for banana and plantain research, 2001). Plantain is a good source of protein, minerals, vitamins and carbohydrate no matter what form it is consumed. It can be boiled, fried as ripe pulp (dodo), fried unripe (chips) and into processed form such as plantain flour. Plantains are particularly high in vitamin B₆ and contain moderate levels of folates, niacin and thiamin (Bhaskar, 2011).

Banana (*Musa sapientum*) is a tropical and sub-tropical fruit that is consumed globally (Alkarhi *et al.*, 2010). Bananas vary in height depending on the variety and growing condition. They are able to protect themselves from the abiotic stress caused by intense sunshine and high temperature by increasing their antioxidant ability (Haripyaree *et al.*, 2010; Kanazawa & Sakakibara, 2000). Banana fruit are rich in minerals (Potassium, magnesium and phosphorus), dietary fiber and various antioxidants such as Vitamin C, Vitamin E (Kanazawa & Sakakibara 2000).

Plantain and Banana can be used in various ways which include; medical uses, industrial uses and as food. They contain about 2.3g of fiber and this helps for normal bowel movements, thereby reducing constipation problems (Bhaskar, 2011).

In Nigeria, four main types of plantain are available with distribution strictly based on their bunch characteristics. These are the Horn type, French type, the French-horn type and False horn type. The False horn type is the most widely distributed because of its ability to tolerate poor soil conditions and they are grown in Ondo, Ogun, Oyo, Cross river, Imo and Abia States (Idachaba, 2000). There are mainly three types of bananas in Nigeria; Cooking banana, English and Native bananas.

Antioxidants

Antioxidants are substances that can prevent and counteract the damaging effect of oxidation in body tissues due to the oxidative damage of lipids, proteins and nucleic acids caused by reactive oxygen species (Scheibmeir *et al.*, 2005). Antioxidants scavenge free radicals by inhibiting initiation and also by breaking chain propagation. They suppress the formation of free radicals by binding to metal ions, by reducing hydrogen peroxide and by quenching superoxide and singlet oxygen (Lim *et al.*, 2007). Glutathione is an important water soluble antioxidant. It is synthesized from the amino acids glycine, glutamate and cysteine. Glutathione reacts with toxic oxygen radicals to form

glutathione radicals and subsequently glutathione disulfide (GSSG), thereby protecting against oxidative damage to DNA lipids and proteins. (Schafer *et al.*, 2001). Carotenoids are pigmented compounds that are synthesized by plants (Mattea *et al.*, 2009). Carotenoids such as lycopene and β -carotene contribute to the beneficial properties of fruits and vegetables in preventing human diseases including cardiovascular disease, cancer and other chronic diseases (Rao & Rao, 2007). Lycopene as an antioxidant acts as a free radical scavenger; counteracting the damaging effects of oxidative stress (Wayne *et al.*, 2002). Lycopene reduces the levels of oxidized low density lipoproteins (LDL) and total cholesterol, thereby lowering the risk of cardiovascular diseases (Rao & Rao, 2007). β -carotene is a lipophilic carotenoid that has provitamin A activity due to the presence of terminal β -ionone rings in its structure (Bast & Haenen, 2002). β -carotene is an efficient singlet oxygen quencher and prevents the formation of singlet oxygen by quenching excited triplet sensitizers (Demmig & Adams, 2002). Vitamin E also known as tocopherol is a fat-soluble vitamin and an important antioxidant with antioxidant property (Herrera & Barbas, 2001). Lycopene, β -carotene, glutathione and vitamin E are important antioxidants that are very beneficial in countering the harmful effect of free radicals or reactive oxygen species (ROS) (Pallavi *et al.*, 2012).

Phytochemical

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventing properties. They are non-nutritive because they do not fall under the categories of carbohydrates, proteins, vitamins, fat or minerals in food classification and are not necessary for sustaining life. Although they are non-nutritive they are however beneficial to our health. Phytochemicals are now associated with the prevention and/or treatment of at least four of the leading causes of death in some regions of the world, which are, cancer, diabetes, cardiovascular disease and hypertension (Liu, 2003). They are involved in many processes including those that help prevent cell damage, prevent cancer cell replication and decrease cholesterol levels (Meskin *et al.*, 2002). They include Alkaloids, saponins, phenols, flavonoids, and tannins.

The aim of this study therefore, was to determine the levels of glutathione, carotenoids, vitamin E, phytochemicals, which are very important natural antioxidants in various cultivars of plantain and banana and to also determine the antioxidant activity of these fruits during ripening.

MATERIALS AND METHODS

Mature fresh samples of *Musa species* were used for this study. They include two cultivars of *Musa paradisiaca* (French and False horn, which are locally known as Bini and Auchi plantains, respectively) and three cultivars of *Musa sapientum* (Dwarf Cavendish, Bluggoe cacambou, Gros michel: mutant, which are locally known as English, Cooking and Native bananas, respectively). They were purchased in their unripe stage from a farm in Benin City, Nigeria and allowed to ripen naturally at ambient temperature ($30 \pm 2^\circ\text{C}$) in a ripening chamber.



Fig. 1a. *MUSA PARADISIACA* CV. FRENCH (BINI PLANTAIN) that was used in this study with its different ripening stages



Fig. 1b. *MUSA PARADISIACA* CV. FALSE HORN (AUCHI PLANTAIN) that was used in this study with its different ripening stages



Unripe

Ripe

Overripe

Fig. 1c. *MUSA SAPIENTUM* CV. DWARF CAVENDISH (ENGLISH BANANA) that was used in this study with its different ripening stages



Unripe

Ripe

Overripe

Fig. 1d. *MUSA SAPIENTUM* CV. BLUGGOE CACAMBOU (COOKING BANANA) that was used in this study with its different ripening stages



Unripe

Ripe

Overripe

Fig. 1e. *MUSA SAPIENTUM* CV. GROS MICHEL: DWARF MUTANT (NATIVE BANANA) that was used in this study with its different ripening stages

Estimation of Antioxidant activity

Extraction of antioxidant was carried out by the method of Sulaiman *et al.*, (2011). The plantain or banana sample was washed, diced and blended with a mortar and pestle to form slurry. Ten (10) grams of each of the plantain or banana slurry was extracted separately with 100 ml Aqueous, 100 ml methanol and stirred on a magnetic stirrer for 30 minutes. The extract was filtered using cheese cloth and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected for the estimation of antioxidant activity. The antioxidant activity was carried out by DPPH Free Radical Scavenging Assay as described by Brand & Williams (1995) with slight modification. 7.5 ml of 0.3 mM DPPH in methanol were mixed with 2.5 ml of plantain or banana extract and incubated at 37 °C for 30 minutes. The decrease in absorbance value was measured at 517 nm with a spectrophotometer. In the blank (Control) the plantain or banana extract was replaced with distilled water. The assay was carried out in triplicate, the percentage scavenging inhibition or activity was determined and compared with that of ascorbic acid, which was used as a standard antioxidant.

Estimation of Phytochemical constituents of plantain and banana**Tannin determination**

Tannins were determined based on the method of Van-Burden & Robinson method (1981). 50ml of distilled water was added to 5 g of dried pulverized plantain or banana sample and shaken for 1hr in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipette out into a test tube and mixed with 2ml of 0.1 M FeCl₃ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Flavonoid determination

The flavonoids in the dried pulverized plantain or banana sample were extracted according to the method of Bohm & Kocipai-Abyazan (1994). Ten (10 g) of the plant sample was extracted repeatedly with 100 ml of 80% aqueous-methanol at room temperature. The extract was filtered through Whatman filter paper No 42 (125 mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed until a constant weight was obtained. Aluminum chloride colorimetric method of Chang *et al.* (2002) was used to estimate the flavonoids.

Phenol determination

Total phenol was determined spectrophotometrically. The dried pulverized sample was boiled with 50 ml of ether for 15 min to extract the phenols. 5 ml of the extract was pipette into 50 ml volumetric flask to which 10 ml of distilled water, 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The solution was made up to mark and left to react for 30 min for colour development. The absorbance was measured at 505 nm.

Saponin determination

The method of Obadoni & Ochuko (2001) was used for the determination of saponin in the dried pulverized plantain or banana sample. 100 ml of 20% aqueous-ethanol was added to 20 g of the sample in a conical flask. The mixture was heated over a hot bath for 4 hr at 55°C with continuous stirring. The mixture was filtered and the residue re-extracted with another 200 ml 20% aqueous- ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated after which 60 ml of n-butanol was added. The n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight, the saponin content was calculated as percentage.

Alkaloid determination

Alkaloids were determined by the method of Harborne (1973). Five (5) grams of the dried pulverized of plantain or banana sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, it was covered and allowed to stand for 4 hr. The extract was filtered and concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue obtained was the alkaloid, which was dried and weighed to determine the quantity.

Preparation of extract for the estimation of glutathione

The level of reduced glutathione was estimated by the method of Moron *et al.*, (1979). One gram (1g) of the fresh plantain or banana sample was homogenized in a mortar with acid- washed sand and 5 ml of 5% trichloroacetic acid. The homogenate was immediately acidified by adding 250µl of 25% of trichloroacetic acid to prevent aerial oxidation of glutathione. The homogenate was filtered through a double layer of cheese cloth and was centrifuged at 1000 g for 10 minutes. The supernatant obtained was used for the estimation of glutathione.

Estimation of glutathione

An aliquot of 0.1ml of the supernatant obtained from the extraction of glutathione was cooled on ice and made up to 1 ml with 0.9 ml of 0.2 M sodium phosphate buffer (pH 8.0). 2 ml of freshly prepared 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate buffer was added to the chilled supernatant and the intensity of the yellow colour that was formed was read at 412 nm in a spectrophotometer after 10 min of incubation. The blank (Control) contained 0.1 ml of distilled water, 0.9 ml of 0.2 M sodium phosphate buffer (pH 8.0) and 2 ml of freshly prepared 0.6 mM 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) in 0.2 M sodium phosphate buffer. The amounts of glutathione were extrapolated from a standard calibration curve and the values were expressed as nanomole of glutathione/g fresh weight.

Preparation of extract for the estimation of Vitamin E (Tocopherol)

The level of tocopherol (Vitamin E) was estimated spectrophotometrically by the method of Rosenberg (1992). A sample weighing 2.5 g of the fresh plantain or banana sample was homogenized in a mortar with acid-washed sand and 0.14 ml of 0.05 M sulphuric acid. The volume of the homogenate was finally made up to 50 ml by adding 0.05 M sulphuric acid slowly and was allowed to stand overnight. The content of the flask was shaken vigorously on the next day and filtered through Whatmann No. 1 filter paper. The filtrate was used for the estimation of vitamin E.

Estimation of vitamin E

A measured volume of 3ml each of absolute ethanol and xylene were added to an aliquot of 3ml of the filtrate obtained from the extraction of vitamin E. The mixture was thoroughly mixed for 2 min to obtain a homogenous mixture, which was centrifuged at 1000 g for 10 min. After centrifugation, 2.0 ml of 120 mg/100 ml 2,2'-dipyridyl in propanol were added to 2.0 ml of the xylene layer, which was the supernatant and the absorbance read at 480 nm. 0.66 ml of 120 mg/100 ml ferric chloride in ethanol was added to the reaction mixture and the absorbance was read at 520nm at exactly 30 sec. 1mg/100ml vitamin E and distilled water were used as standard and blank (Control) respectively.

Preparation of extract for the estimation of β-Carotene and Lycopene (Carotenoids)

The levels of β-Carotene and Lycopene (Carotenoids) were estimated by the method of Bortolotti *et al.* (2003). Ten (10) g of the fresh plantain or banana sample was ground in a mortar with acid washed sand. The ground sample was added to 20ml acetone and 30ml hexane contained in a beaker and the content was stirred with a glass rod. The mixture was poured into a separating funnel.

Estimation of β -Carotene and Lycopene (Carotenoids)

The content of the separating funnel (the mixture) was shaken vigorously until the content became homogenous. The mixture was allowed to separate into layers and the top layer was used for the estimation. The absorbance of the top layer was taken at 453nm for β -carotene and 503 nm for lycopene. At 503 nm, lycopene has a maximum absorbance while carotene has only a negligible absorbance. (Zakaria *et al.*, 1979).

Statistical Analysis

Data obtained were expressed as Means $\pm$ SEM for three replicates. One way ANOVA was used to analyze the data, followed by Post Hoc multiple comparison test. Statistical significance was established at $p < 0.01$. Data computation was facilitated using the Statistical Package for Social Sciences (SPSS version 20.0).

RESULTS

The antioxidant activity increased with ripening in the plantain and banana cultivars, with their ripe and overripe stages having the highest values. Methanolic extracts of the plantain and banana cultivars showed higher antioxidant activity than that of aqueous extracts (Figs. 1a - 2c).

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	**20.86 $\pm$ 0.04 ^a	22.86 $\pm$ 0.41 ^a	21.94 $\pm$ 0.01 ^a
Methanol	29.22 $\pm$ 0.00 ^a	31.65 $\pm$ 0.18 ^b	32.75 $\pm$ 0.00 ^c

Table 1a: *Antioxidant activity of the fruits of *Musa paradisiaca* cv. French (Bini plantain) at various ripening stages

**Mean $\pm$ SEM (n=3)

Means in the same row followed by different letters are significantly different at $p < 0.001$

Means in the same row followed by the same letter are not significantly different at $p < 0.001$

*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits

Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.28 $\pm$ 0.34

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	**20.01 $\pm$ 0.04 ^a	24.71 $\pm$ 0.00 ^b	25.43 $\pm$ 0.00 ^c
Methanol	29.23 $\pm$ 0.04 ^a	33.07 $\pm$ 0.00 ^b	33.22 $\pm$ 0.00 ^b

Table 1b: *Antioxidant activity of the fruits of *Musa paradisiaca* cv. False Horn (Auchi plantain) at various ripening stages

**Mean $\pm$ SEM (n=3)

Means in the same row followed by different letters are significantly different at $p < 0.001$

Means in the same row followed by the same letter are not significantly different at $p < 0.001$

*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits

Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.68 $\pm$ 0.06

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	**21.37 $\pm$ 0.29 ^a	22.72 $\pm$ 0.04 ^a	22.72 $\pm$ 0.04 ^a
Methanol	30.15 $\pm$ 0.00 ^a	33.07 $\pm$ 0.00 ^b	32.07 $\pm$ 0.00 ^c

Table 2a: *Antioxidant activity of the fruits of *Musa sapientum* cv. Dwarf cavendish (English banana) at various ripening stages

**Mean $\pm$ SEM (n=3)

Means in the same row followed by different letters are significantly different at $p < 0.001$

Means in the same row followed by the same letter are not significantly different at $p < 0.001$

*Antioxidant activity was determined by the % DPPH scavenging activity of the

fruits. Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.68 $\pm$ 0.06

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	** 20.67 $\pm$ 0.04 ^a	23.29 $\pm$ 0.10 ^b	23.52 $\pm$ 0.0 ^b
Methanol	29.39 $\pm$ 0.05 ^a	32.60 $\pm$ 0.00 ^b	33.44 $\pm$ 0.04 ^c

Table 2b: *Antioxidant activity of the fruits of *Musa sapientum* cv. Bluggoe cacambou (Cooking banana) at various ripening stages **Mean $\pm$ SEM (n=3)

Means in the same row followed by different letters are significantly different at $p < 0.001$

Means in the same row followed by the same letter are not significantly different at $p < 0.001$

*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits

Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.68 $\pm$ 0.06

Extraction Solvent	Unripe fruit	Ripe fruit	Overripe fruit
Aqueous	** 21.37±0.29 ^a	21.37±0.29 ^a	23.67±0.00 ^b
Methanol	30.15±0.00 ^a	32.39±0.04 ^b	33.73±0.04 ^c

Table 2c: *Antioxidant activity of the fruits of *Musa sapientum* cv. Gros michel: dwarf mutant (Native banana) at various ripening stages **Mean ± SEM (n=3)

Means in the same row followed by different letters are significantly different at $p < 0.001$

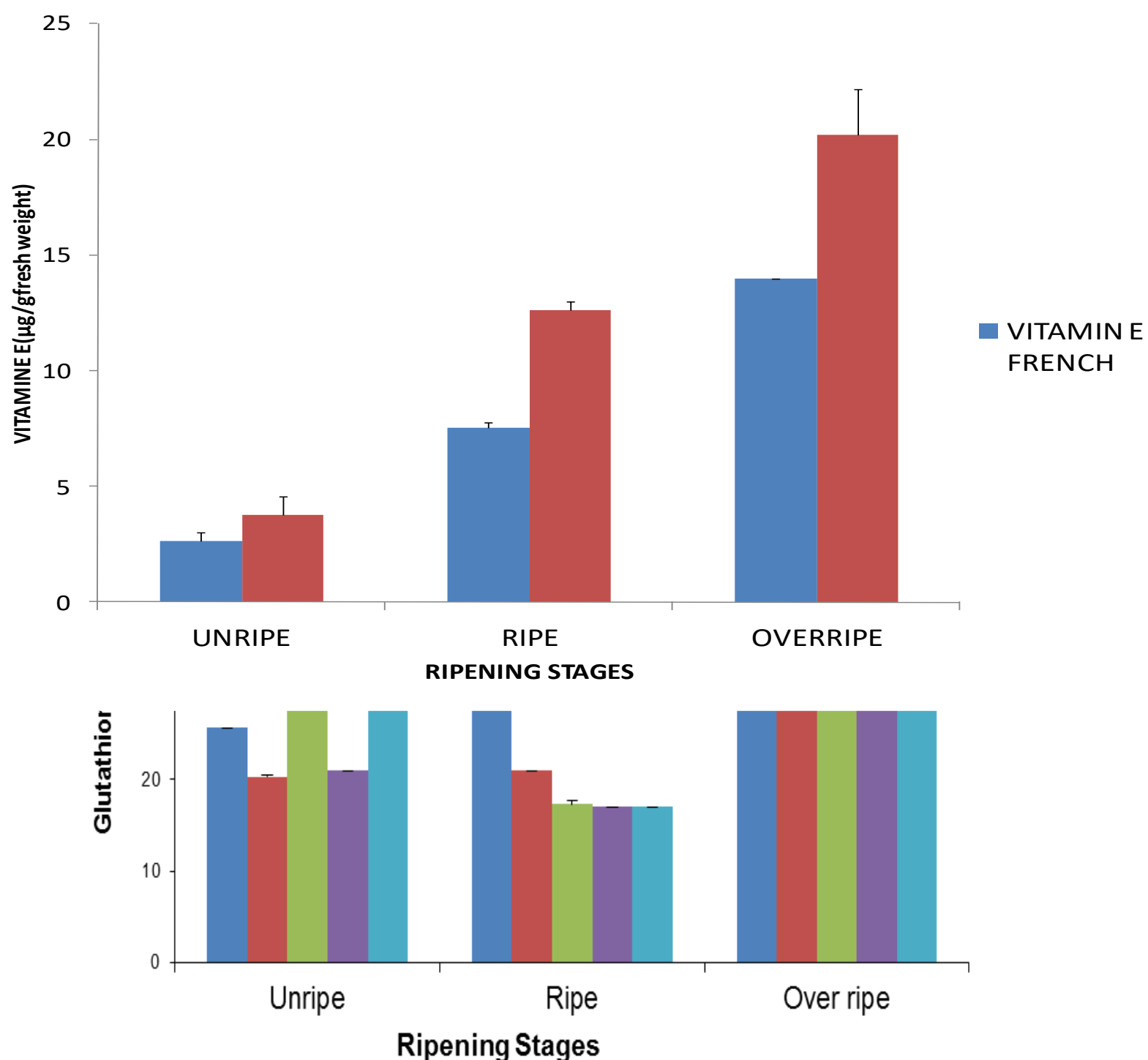
Means in the same row followed by the same letter are not significantly different at $p < 0.001$

*Antioxidant activity was determined by the % DPPH scavenging activity of the fruits

Antioxidant activity of ascorbic acid (Standard antioxidant) was 23.68±0.06

Glutathione, vitamin E, carotenoids and phytochemicals are major contributors to antioxidant status, due to their protective roles in scavenging free radicals or reactive oxygen species (ROS) that induce oxidative damage, which results in degenerative diseases. In this study, significant increase ($p < 0.001$) was observed in the levels of glutathione in all plantain and banana cultivars during ripening, with the overripe stage of each cultivar having the highest glutathione level (Fig. 2).

Fig. 2. Glutathione levels of *Musa sapientum* cvs. *Bluggoe cacambou*, Dwarf cavendish, Gros Michel: dwarf mutant (Cooking, English and Native bananas) and *Musa paradisiaca* cvs. French and False Horn (Bini and Auchi plantains) during different ripening stage



Vitamin E levels showed significant increase ($p < 0.001$) from the unripe to the overripe stage in all the plantain and banana cultivars (Figs. 3a and 3b). **Fig. 3a.** Vitamin E levels of *Musa paradisiaca* cvs. French and False Horn (Bini and Auchi plantains) during different ripening stages.

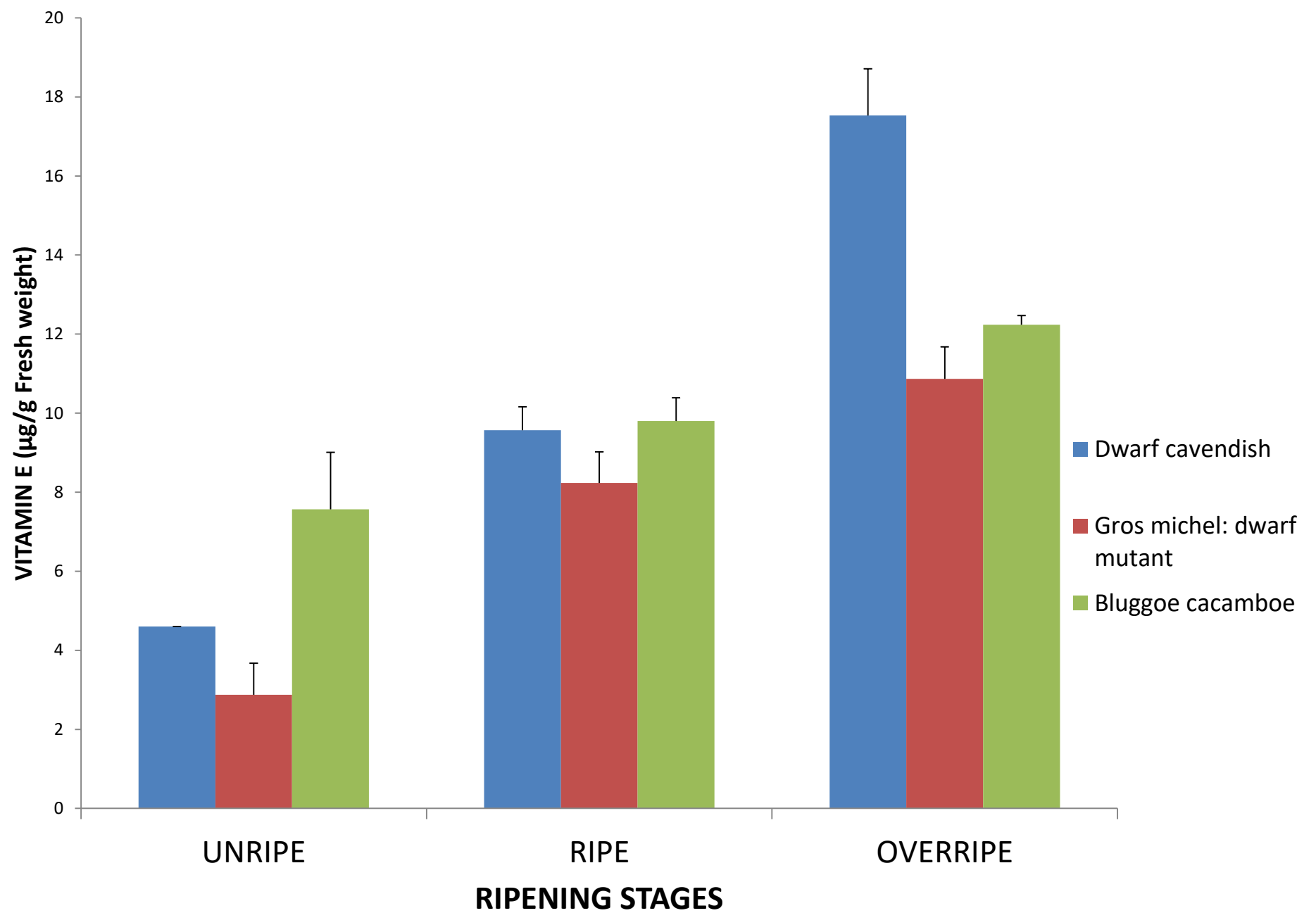


Fig. 3b. Vitamin E levels of *Musa sapientum* cvs. Dwarf cavendish, Gros Michel: dwarf mutant and *Bluggoe cacambou* (English, Cooking and Native bananas) during different ripening stages.

Lycopene levels of the plantain and banana cultivars also increased significantly ($p < 0.001$) with ripening. β -carotene was not detected in the banana cultivars during ripening. It was also not detected in the French plantain however; a negligible level was detected in the unripe stage of the False Horn plantain.

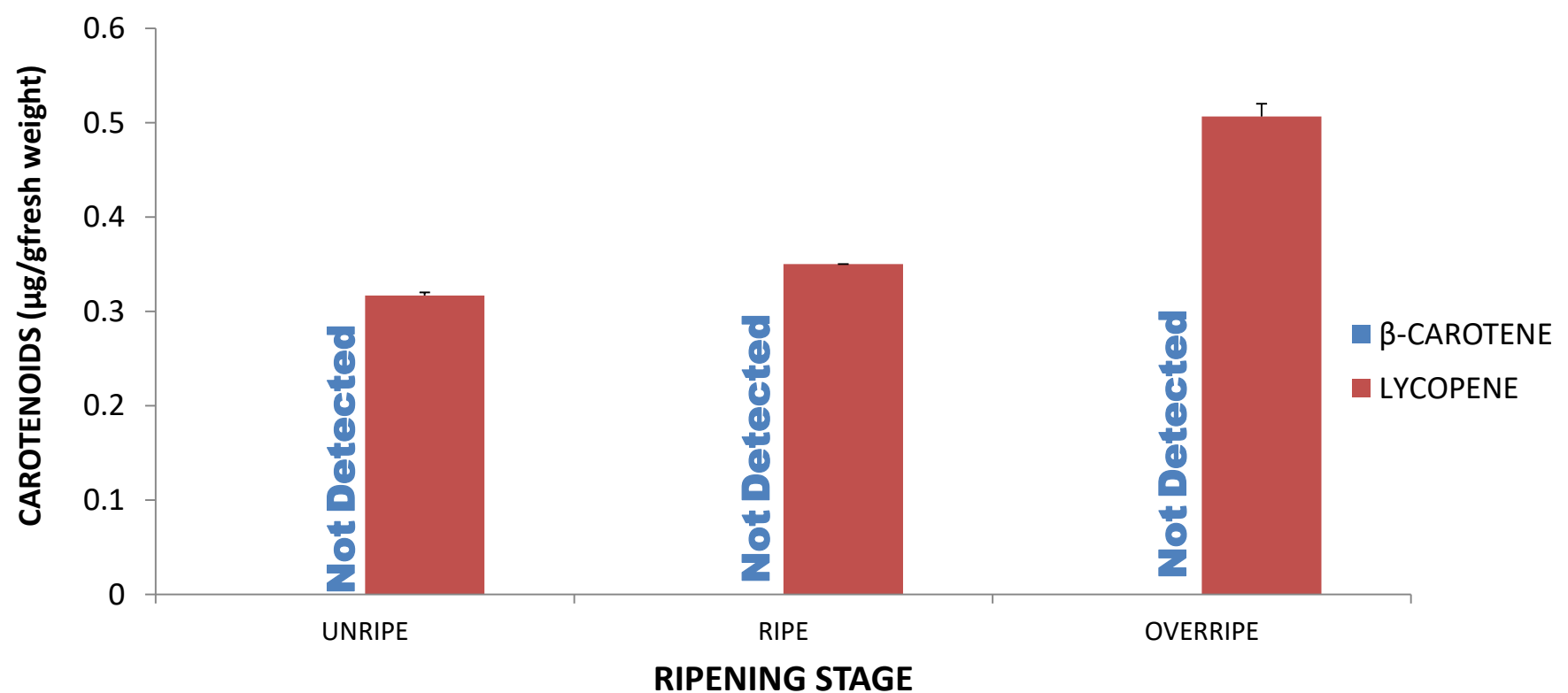


Fig. 4a. Carotenoid levels of *Musa paradisiaca* cv. French (Bini plantain) during ripening

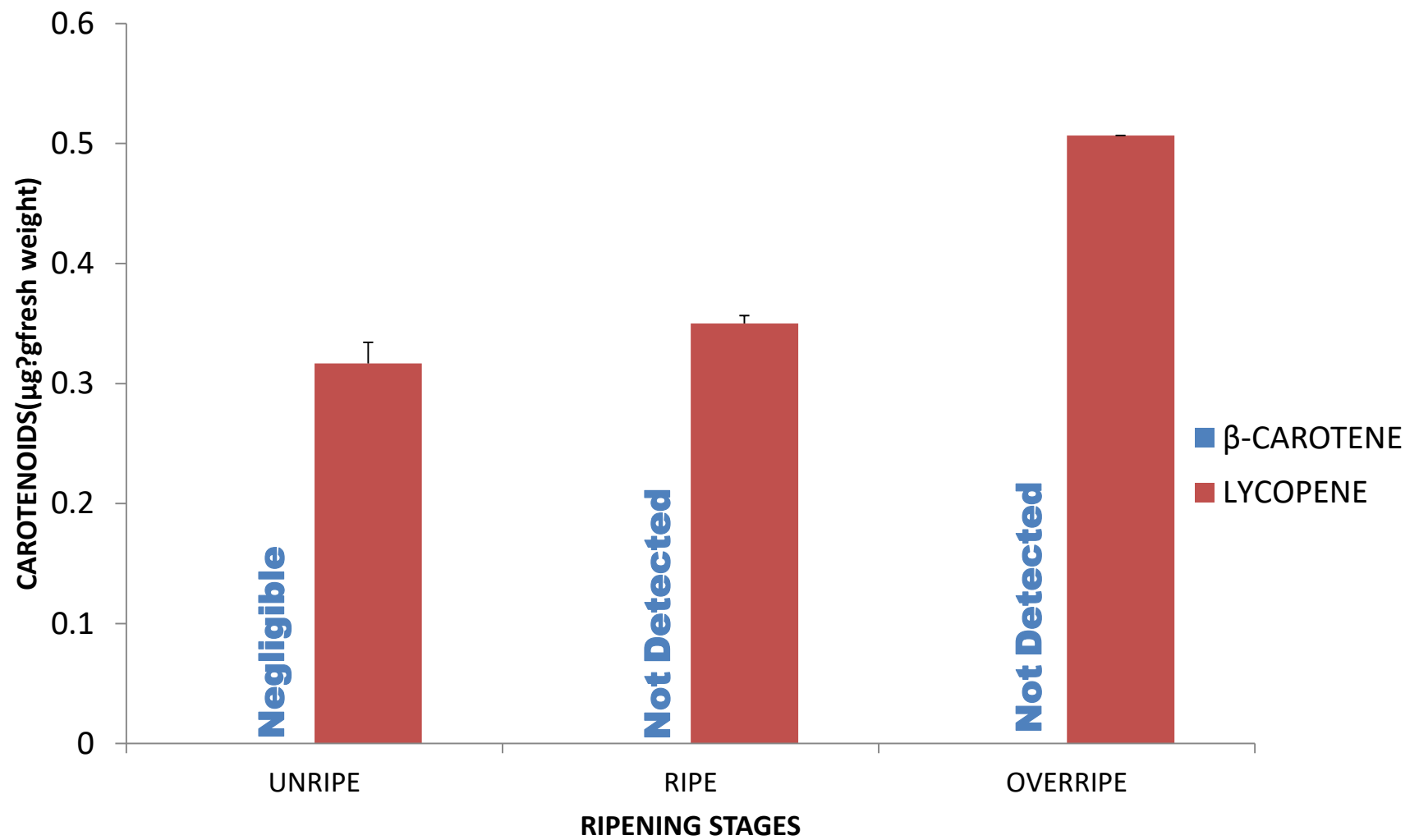


Fig. 4b. Carotenoid levels of *Musa paradisiaca* cv. False Horn (Auchi plantain) during ripening

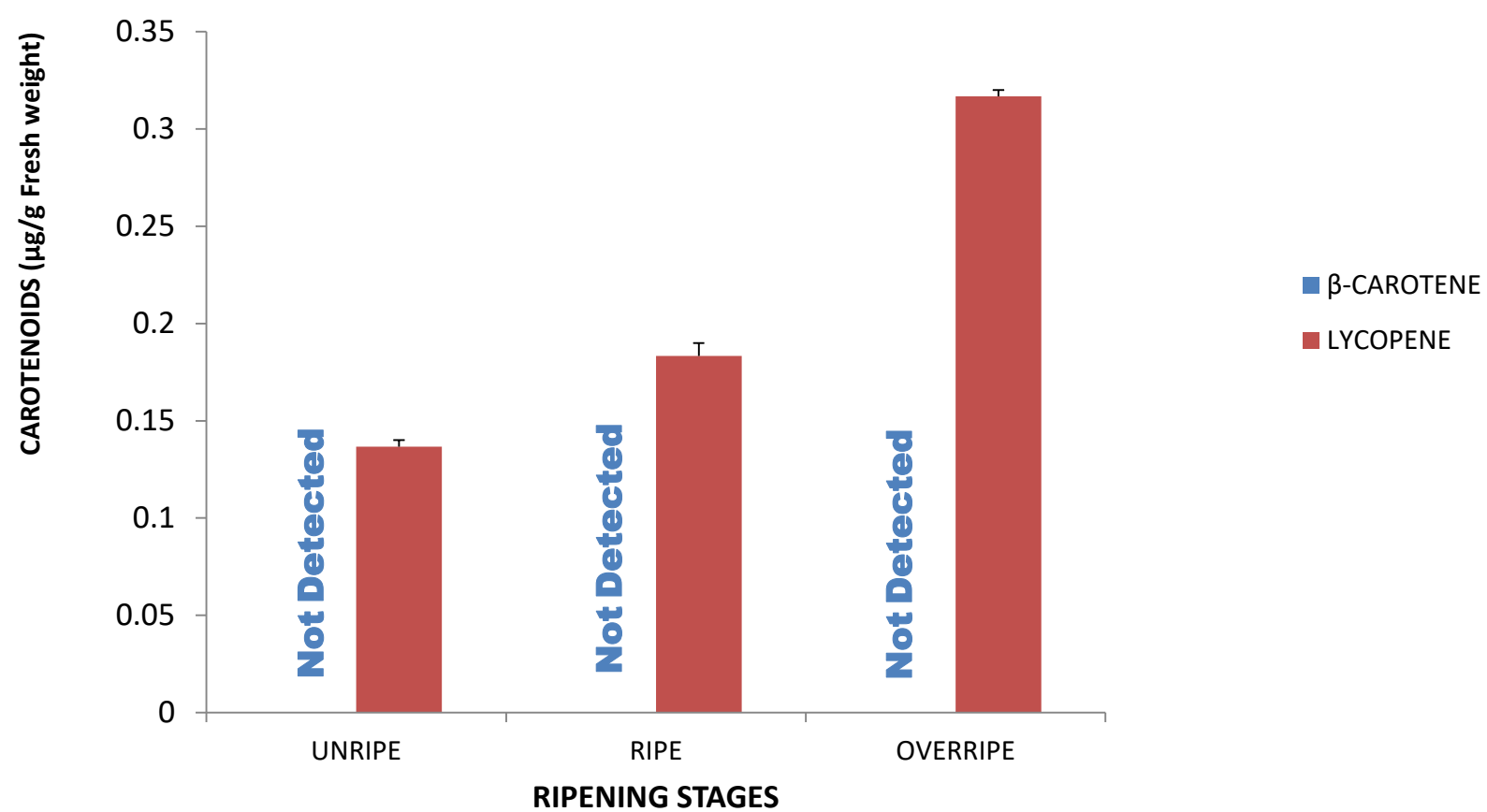


Fig. 4c. Carotenoid levels of *Musa sapientum* cv. Dwarf Cavendish (English banana) during ripening

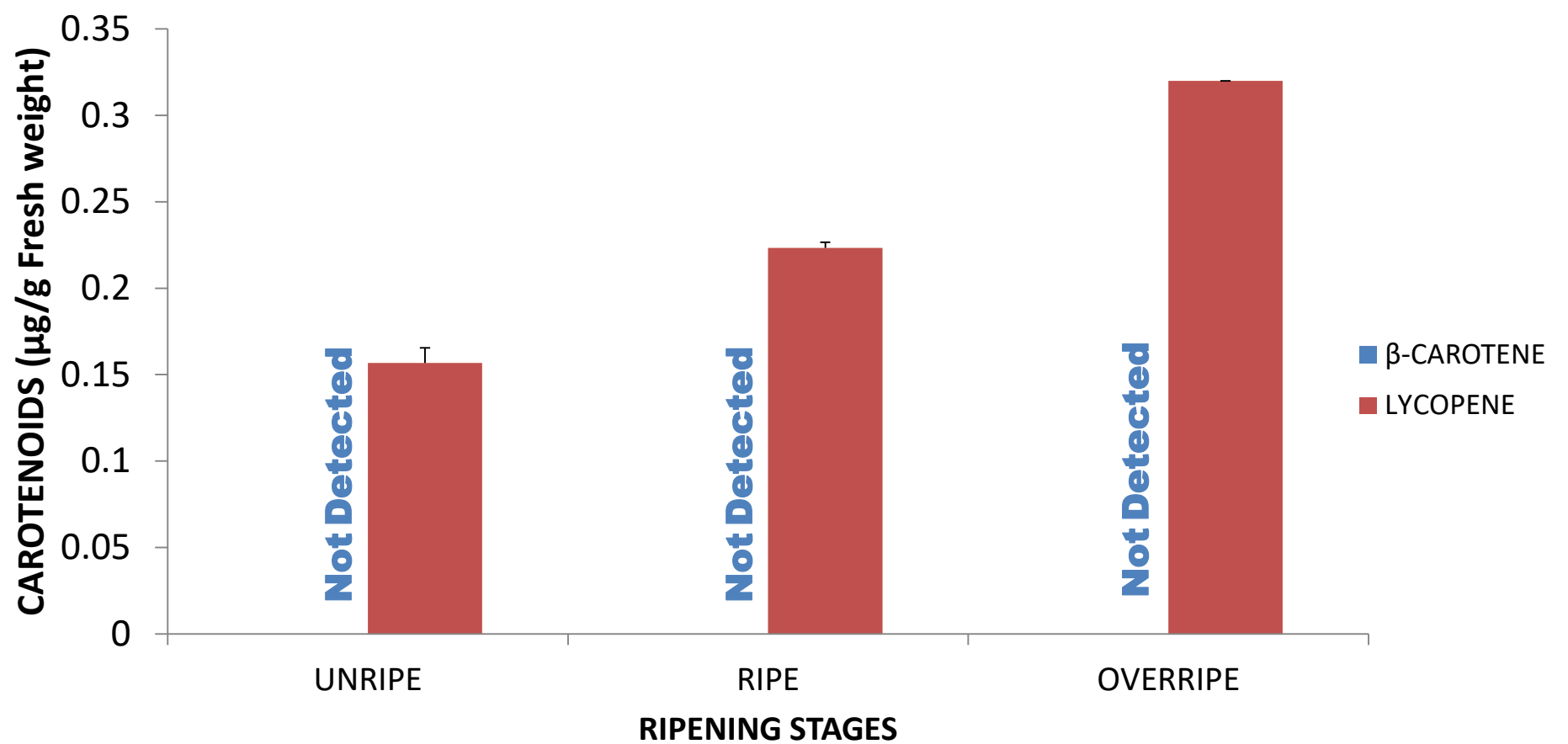


Fig. 4d. Carotenoid levels of *Musa sapientum* cv. Gros Michel: dwarf mutant (Native banana) during ripening

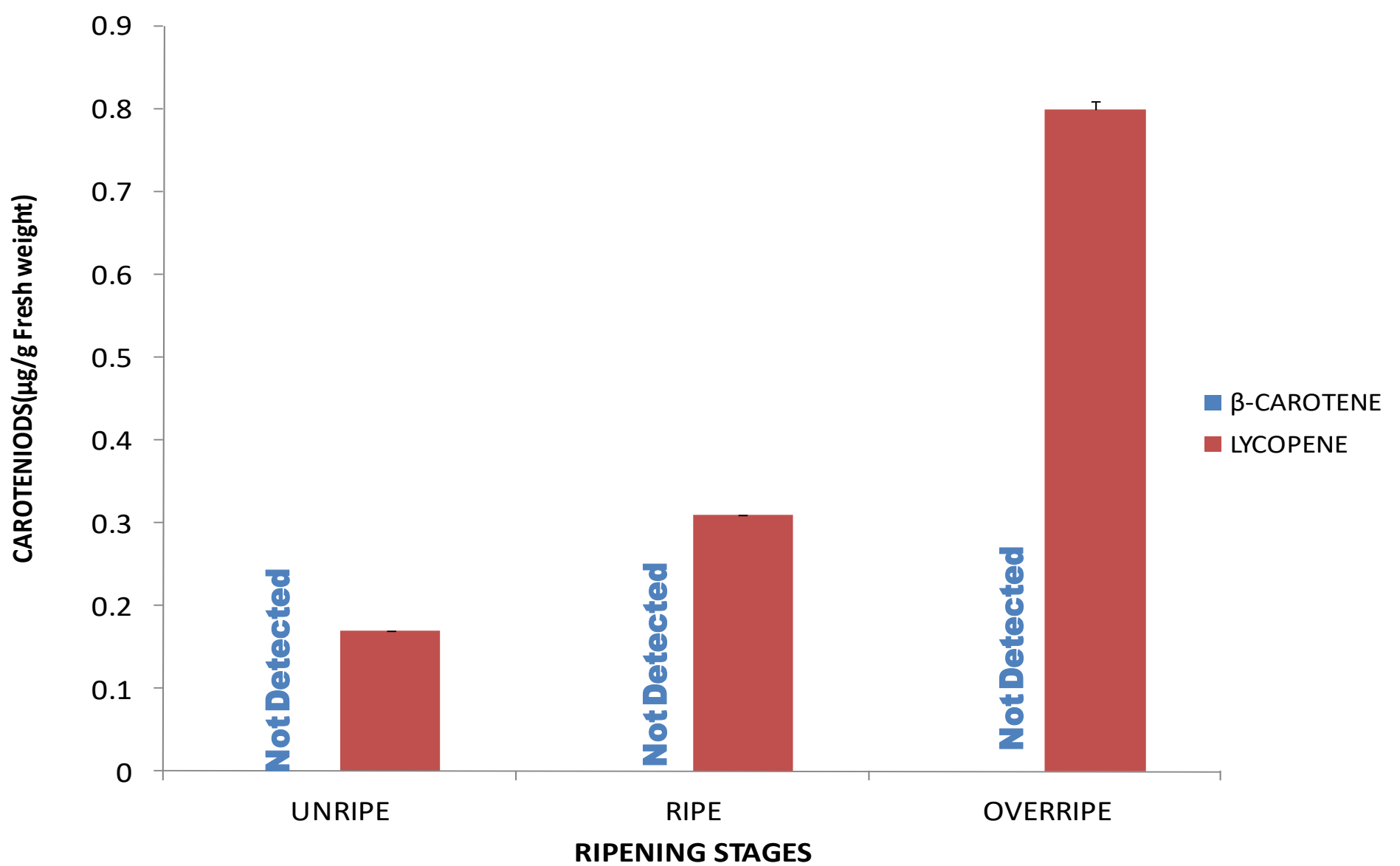


Fig. 4e. Carotenoid levels of *Musa sapientum* cv. Bluggoe cacambou (Cooking banana) during ripening

Phytochemical screening of the plantain and banana cultivars showed decreased levels of tannins, phenols and alkaloids but increased levels of saponins and flavonoids as ripening progressed. However a decrease in the level of saponin was observed in the cooking banana (*Musa sapientum* cv. Bluggoe cacambou) Figs 5a – 5e.

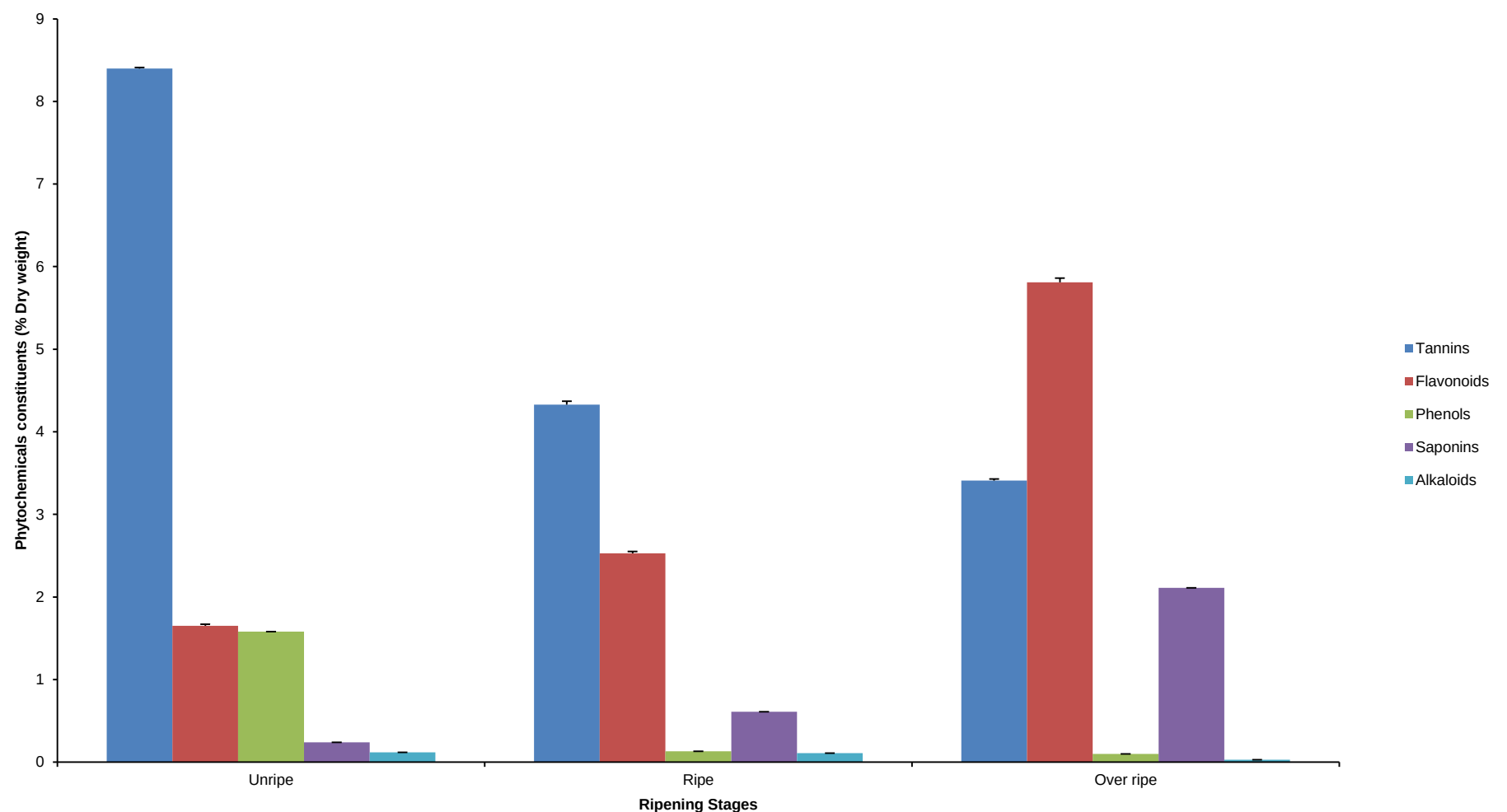


Fig. 5a Phytochemicals constituents of *Musa paradisiaca* cv. False Horn (Auchi plantain) at various ripening stages

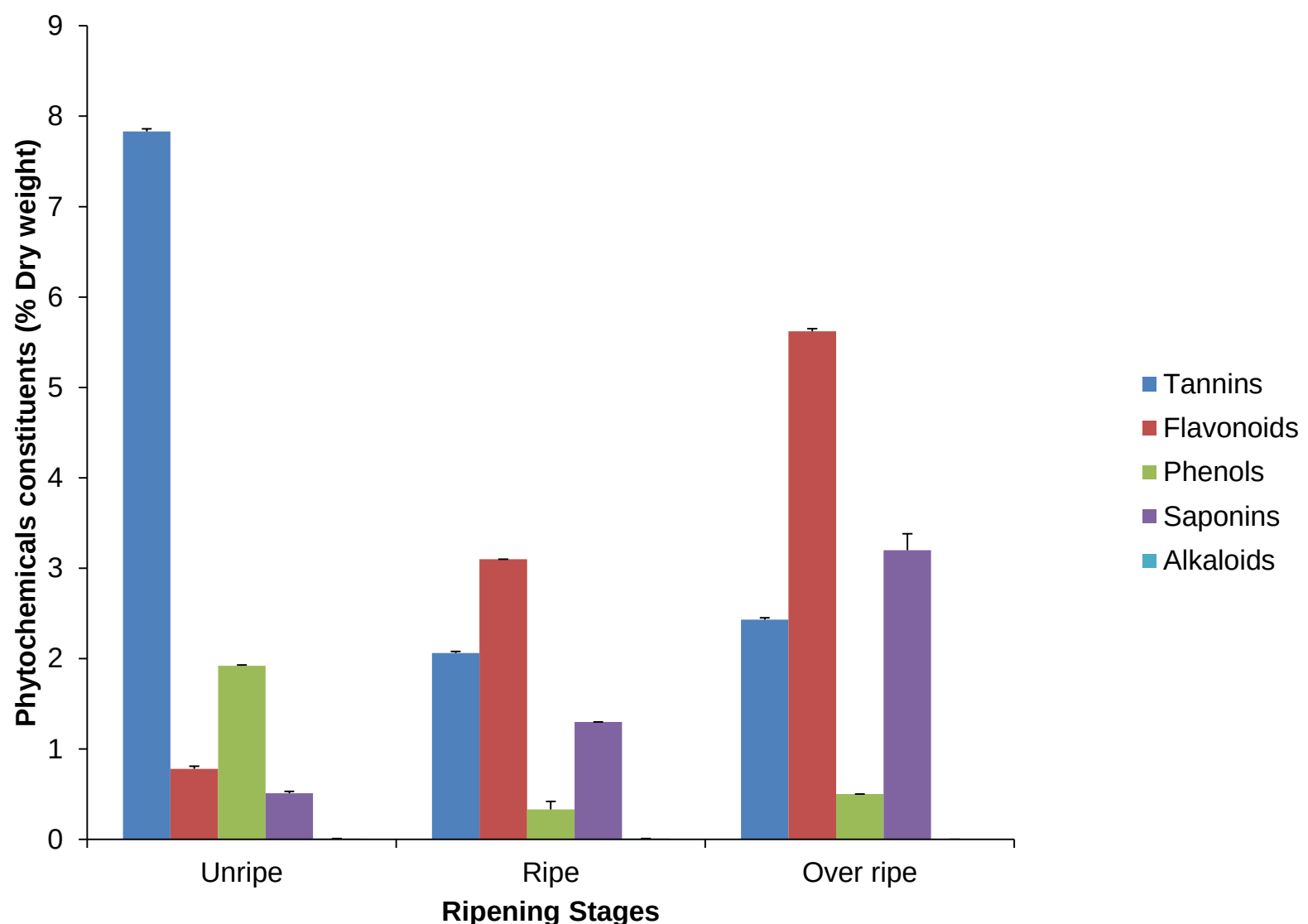


Fig. 5b. Phytochemical constituents of *Musa paradisiaca* cv. French (Bini plantain) at various ripening stages

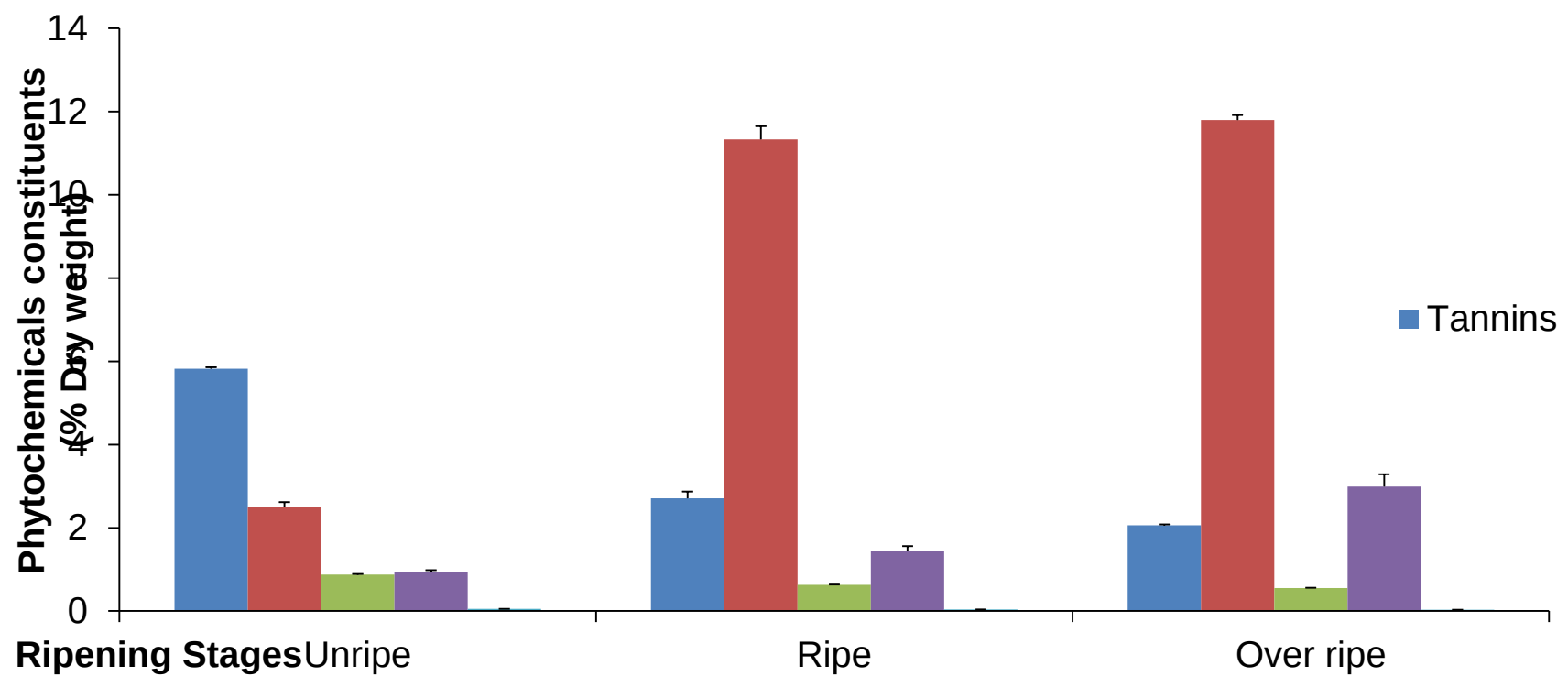
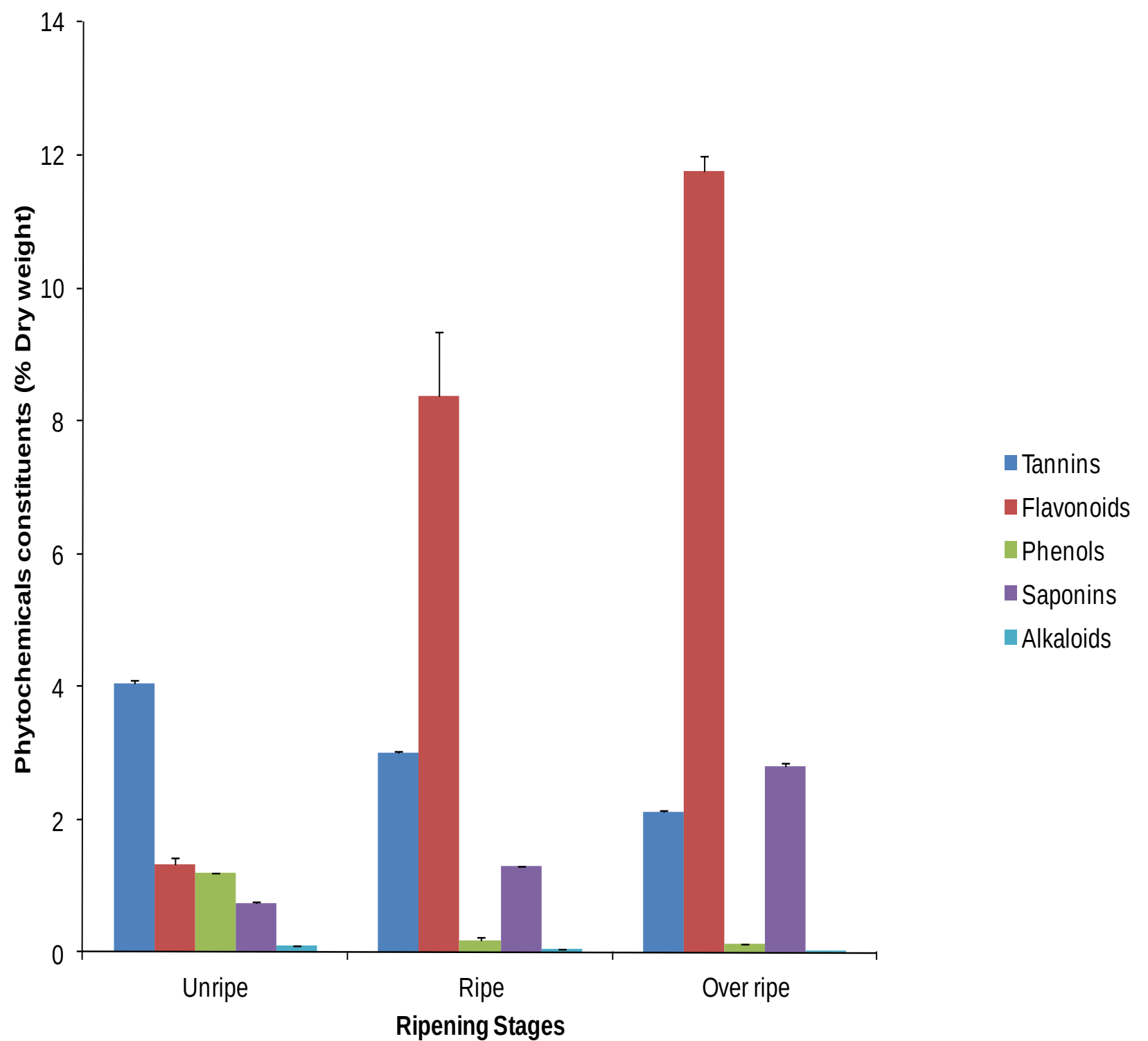


Fig. 5c. Phytochemical constituents of *Musa sapientum* cv. Dwarf Cavendish (English banana) at various ripening stages

Fig. 5d. Phytochemical constituents of *Musa sapientum* cv. Gros Michel: dwarf mutant (Native banana) at various ripening stages



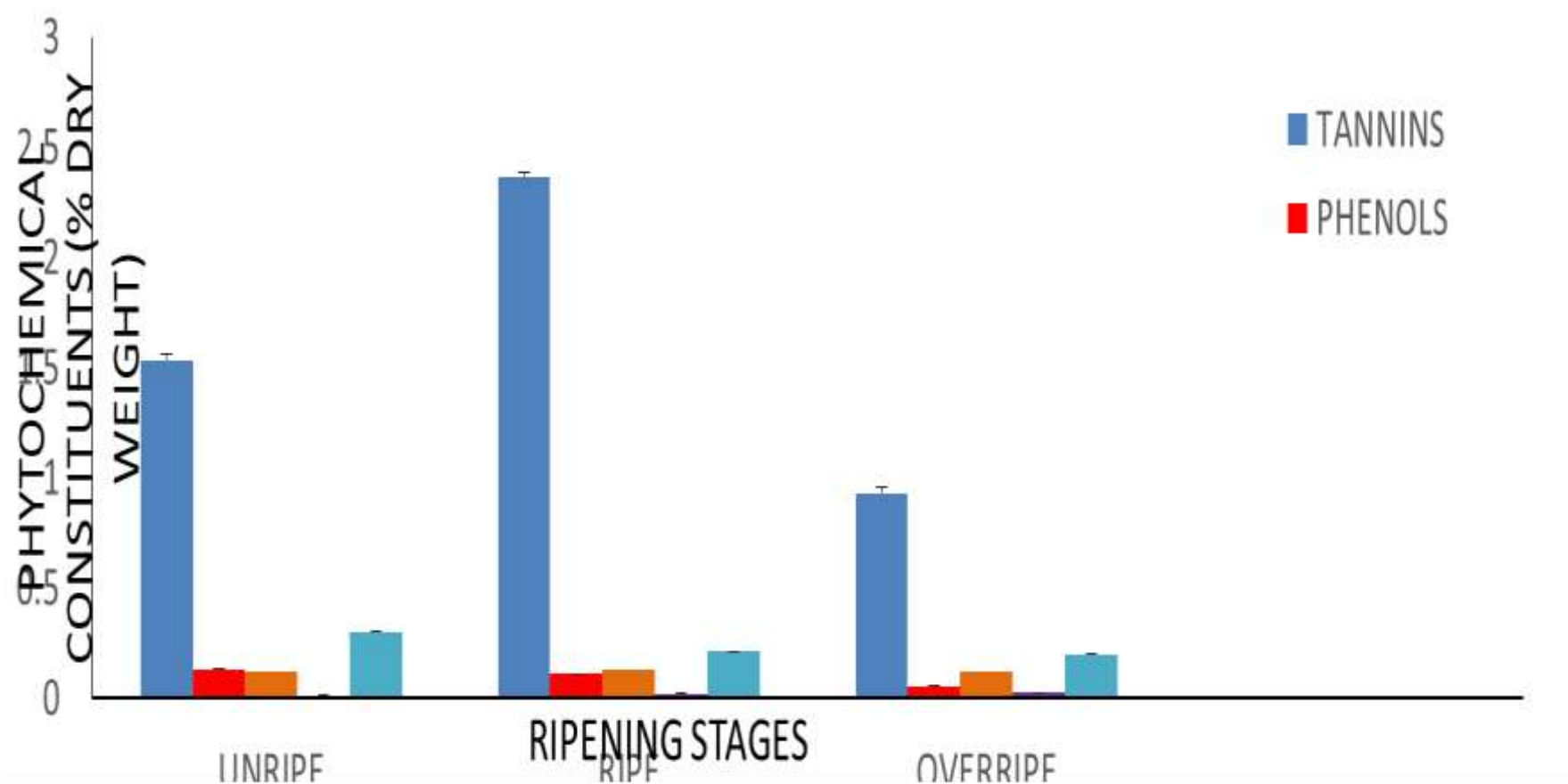


Fig. 5e. Phytochemical constituents of *Musa sapientum* cv. Bluggoe cacambou (Cooking banana) at various ripening stages

DISCUSSION

Fruit ripening is the final stage of development of a fruit, which involves a series of physiological and biochemical events leading to changes in colour, flavour, aroma and texture that makes the fruit both attractive and tasty. In general, the fruit becomes sweeter, less green, more edible and softer as it ripens (Suman *et al.*, 2011). In this study, the antioxidant activity, phytochemical and antioxidant levels of two cultivars of *Musa paradisiaca* (cvs. French and False Horn also known as Bini and Auchi plantains) and three cultivars of *Musa sapientum* (cvs. Dwarf Cavendish, Gros Michel: dwarf mutant and Bluggoe cacambou also known as English, Native and Cooking bananas) at various ripening stages were determined.

Significant increase ($p < 0.001$) was observed in the level of the antioxidant glutathione in all the plantain and banana cultivars during ripening, with the overripe stage of each cultivar having the highest glutathione level. Glutathione is one of the major contributors to the antioxidant status in cells, due to its protective role in scavenging free radicals and preventing oxidative damage of nucleic acids, proteins and lipids (Moskaug *et al.*, 2005). The significant increase ($p < 0.001$) observed in the level of glutathione in these cultivars of *Musa paradisiaca* and *Musa sapientum* during ripening, correlates with that of Lizebeth (2012) who also reported a significant increase in glutathione level in Habenero pepper (MR8H) during ripening. Agoreyo *et al.* (2013) had also reported a significant increase in glutathione level during ripening in *Solanum melongena* (oval variety) and *Solanum aethiopicum*. Jimenez *et al.* (2002) also reported a significant increase in glutathione ripening in tomato fruits. The involvement of glutathione cycle during tomato ripening indicated that the anti-oxidative system plays a fundamental role in the ripening of tomato fruit (Jimenez *et al.*, 2002).

The level of the carotenoid, lycopene increased significantly ($p < 0.001$) with ripening in all the plantain and banana cultivars in this study, but the carotenoid, β -carotene was not detected in the banana cultivars and French plantain during ripening. However, a negligible level of β -carotene was detected in the unripe stage of the False Horn plantain. The level of vitamin E, a tocopherol also showed a significant increase ($p < 0.001$) from the unripe to the overripe stage in all the plantain and banana cultivars that were used in this study.

Carotenoids as well as tocopherols are known to be efficient antioxidants and are capable of scavenging reactive oxygen species generated during oxidative stress (Stahl *et al.*, 2000). Lycopene, which is a carotenoid is one of the most popular pigments accepted by the food industry as a food additive. Biologically, lycopene acts as a singlet oxygen and peroxy radical scavenger (Stahl and Sies, 2003). From this study, lycopene levels of the plantains and bananas that were used increased significantly during ripening, which is similar to the increase in the level of lycopene that was reported for *Maradol papaya* during ripening (Rivera -pastrana *et al.*, 2010). Bramley (2002) also reported a significant increase in carotenoid levels in tomato during ripening due to the accumulation of lycopene. Increase in lycopene in two varieties of *Carica papaya* (local and agric pawpaw) during ripening has also been reported by Agoreyo *et al.* (2013). Although, β -carotene was however not detected in the French plantain and the bananas that were used in this study but in the False Horn plantain where it was detected only in the unripe stage, its level was negligible. Tomatoes have also been reported to contain high level of lycopene and low level of β -carotene (Kokuzue & Friedmann, 2003).

The level of vitamin E in mango fruit has been reported to increase during ripening (Rajesh *et al.* 2011), which is similar to the increase in vitamin E that was observed in this study in all the plantain and banana cultivars during ripening. Agoreyo *et al.* (2013) has also observed an increase in vitamin E during ripening of eggplants (*Solanum melongena* (oval variety) and *Solanum aethiopicum* (round variety)). The antioxidants levels of plantains and bananas in this study correlated with the reports from previous research studies done with pawpaw, tomatoes, mango and egg plants (Kokuzue & Friedmann, 2003; Rivera-pastrana *et al.*, 2010; Agoreyo *et al.* 2013).

Antioxidant activity of plantain and banana cultivars that were used in this study, increased as ripening progressed with their ripe and overripe stages having the highest values. This correlates with the antioxidant activity of red raspberries, in which the highest antioxidant activity was noticed in the ripe and overripe stages (Wang & Lin, 2000). Cherry tomatoes have been reported to exhibit highest carotenoids and antioxidant activity using aqueous extraction (Raffo *et al.*, 2002), while Jacob & Shen, (2011) have also reported the antioxidant

activity of the aqueous extract of *Clerodendrum Serratum* seasonal leafy vegetable. In this study however, the methanolic extracts of the plantain and banana cultivars showed higher antioxidant activity than that of aqueous extracts.

Phytochemical screening of the plantain and banana cultivars showed decreased levels of tannins, phenols, alkaloids but increased levels of saponins and flavonoids as ripening progressed. The decrease in phenol content with ripening agreed with studies by Oladele & Khokhar (2011) and Ibukun *et al.* (2012), who also reported that phenolic content decreased as plantain fruit ripened. It should be noted that the decrease in alkaloid content from unripe to ripe stage was not a significant one ($p > 0.01$).

CONCLUSION

The ripe and over ripe stages of plantains and bananas that were used in this study contained more antioxidant properties which could help to scavenge free radicals and prevent diseases in humans. They therefore, serve as important and natural sources of antioxidants. The consumption and addition of plantain and banana to diets could help to increase the ability of the body to counteract the effect of oxidative damage, irrespective of the cultivar. Also, consumption of these fruits in all the ripening stages have great health benefits because they are important sources of antioxidants, which help to scavenge free radicals or reactive oxygen species (ROS) that are deleterious to health. Plantains are not inferior to bananas as sources of antioxidants and in terms of antioxidant activity, thus their consumption not only regionally in some countries but all over the world is recommended.

REFERENCES

- Agoreyo, B.O., Okhihie, O. and Agoreyo, F.O (2013). Carotenoids, Glutathione and Vitamin E contents of eggplant (*Solanum spp.*) during ripening. *Nigerian Journal of Pharmaceutical & Applied Science Research*, **2**:41-48
- Alkarkhi, A.F., Ramlis S., Yeoh S.Y and Easa, A.M. (2010). Physiochemical properties of banana peel flour as influence by variety and stage of ripening. *Asian Journal of Food Agro Industry*, **3**:349-362.
- Bast, A. and Haenen, G.R.M.M. (2002). The toxicity of antioxidants and their metabolites. *Environmental Toxicology and Pharmacology*, **11**:251-258
- Bhaskar, J.J. (2011). Banana (*Musa sp. varelakki bale*) Flower and Pseudostem: *J Agric Food Chem.*
- Biesalski, H. (2008). Water, Electrolytes and Micronutrients: Phytochemicals. The European Society for Clinical Nutrition and Metabolism (ESPEN) LLL Program.
- Bortolotti, S., Boggio, S.B., Delgado, L., Orellano, E.G. and Valle, E.M. (2003). Different induction patterns of glutamate metabolizing enzymes in ripening fruits of the tomato mutant green flesh. *Physiologia Plantarum*, **119**:384-391.
- Bramley, P.N. (2002). Regulation of carotenoid formation during tomato fruit ripening and development. *Journal of Experimental Botany*, **53**:2107-2113.
- Demmig-Adams, B. and Adams, W. W III. (2002). Antioxidants in photosynthesis and human nutrition. *J. Science*, **298**:2149–2153.
- Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E. and Willett, W. C. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute*, **92**:1812–1823.
- Haripryee, A., Guuneshor, K. and Damayant, M. (2010). Evaluation of Antioxidant properties of some wild edible fruits extract by cell free assays. *Electronic Journal of Environmental, Agriculture and Food Chemistry*, **9**: 345 – 450.
- Herrera, E. and Barbas, C. (2001). Vitamin E : action, metabolism and perspectives. *Journal of Physiology and Biochemistry*, **57**:43-56.
- Ibukun, O. E., Kade, I. J., Oguntoyinbo, T. A., Ogunmoyole, T. and Johnson, O. D. (2012). Effect of ripening on the phytochemical constituents and antioxidant properties of plantain (*Musa paradisiaca*). *Journal of Medicinal Plants Research*, **6**(37):5077-5085.
- Idachaba, F.S (2000). Food Policy in Nigeria. *Agriculture Research Bulletin*, **1**:162.
- International Institute for Banana and Plantain (2001). Annual Report of Plantain in West-Africa.
- Jacob, J. and Shenbagaramen, S. (2011). Evaluation of antioxidant and antimicrobial activities of the selected green leafy vegetable. *Inter. J. Pharma.Tech.Res.* **3**(1): 148-152.
- Jiménez, A., Creissen, G., Kular, B., Firmin, J., Robinson, S., Verhoeyen, M., Mullineaux, P. (2002). Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta*, **214** : 751–758.
- Kanazawa, K. and Sakakibara, H. (2000). High content of dopamine, a strong antioxidant in Cavendish banana. *Journal of Agricultural and Food Chemistry*, **48**: 844-848.
- Kozukue, N. and Friedman, M. (2003). Tomatine, chlorophyll, β -carotene and lycopene content in tomatoes during growth and maturation. *Journal of the Science of Food and Agriculture*, **83**: 195 -200
- Kumar Sampath, P.K., Bhowmik, D., Duraivel, S. and Umadevi, M. (2012). Traditional and Medicinal Uses of Banana. *Journal of Pharmacognosy and Phytochemistry*, **1**(3):51-63.
- Lim, Y.Y., Lim, T.T. and Tee, J.J. (2007). Antioxidant Properties of Several Tropical Fruits: A Comparative study. *Food chemistry*, **103**:1003-1008.
- Liu, R.H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.*, **78**:517S–20S.
- Lizbeth, A.C.C., Ismael, C.C. and Ham-Miranda, L.M. (2012). Determination of Antioxidants in Fruit Tissues From Three Accessions of Habanero Pepper (*Capsicum Chinense* Jacq.). *Journal of Mexican Chemical Society*, **56**:1-10.
- Mattea, F., Martin, A. and Cocero, M. J. (2009). Carotenoid processing with supercritical fluids. *J. Food Eng.*, **93**: 255-265.
- Meskin, M.S., Wayne, R. B., Audra, J. D. and Stanley, T. O. (2002). Phytochemicals In Nutrition and Health. Crc Press. Florida. U.S.A. pp221.
- Moron, M.S., Depierre, J.N. and Mannenik, V.C. (1979). Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, **582** :67-68.
- Moskaug, J.O., Carlsen, H., Myhrstad, M.C.W and Blomhoff, R. (2005). Polyphenols and glutathione synthesis regulation. *The American Journal of Clinical Nutrition*, **81**:277S- 283S.
- Nelson, S.C., Ploetz, R.C. and Kepler, A.K. (2006). *Musa species* (banana and plantain). Species profiles for Pacific Island Agroforestry.
- Obadoni, B.O., and Ochuko, P.O. (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure Applied Science*, **8**:203-208.
- Oladele, E. and Khokhar, S. (2011). Effect of Domestic Cooking on the Polyphenolic Content and Antioxidant Capacity of Plantain (*Musa paradisiaca*) *World Journal of Dairy and Food Sciences*, **6** (2): 189-194.
- Pallavi, S., Ambuj, B.J, Rama, S.D. and Mohammad, P. (2012). Reactive oxygen species, oxidative damage and antioxidative defense conditions. *Journal of Botany*, **2012**:1-26.

- Pari, L. and Umamaheswari, J (2000). Antihyperglycaemic activity of *Musa sapientum* flowers: effect on lipid peroxidation in alloxan diabetic rats. *Phytotherapy Research*, **14**(2): 136-138
- Rajesh, K.S., Sharique, A.A., Pravendra, N. and Vidhu, A. (2011). Activation of ethylene- responsive p-hydroxy phenylpyruvate dioxygenase leads to increased tocopherol levels during ripening in mango, *Journal of Experimental Botany*, **62**:3375-3385.
- Rao, A.V and Rao, L.G. (2007). Carotenoids and human health. *Pharmacological Research*, **55**:207-216.
- Rivera-Pastrana, D.M., Yahia, E.M. and González-Aguilar, G.A. (2010). Phenolic and Carotenoids Profiles of Papaya fruit (*Carica papaya* L.) and their contents under low temperature storage. *J. Sci. Food Agric.*, **90**:2358-2365.
- Rosenberg, H.R. (1992). Chemistry and physiology of vitamins. *Interscience Publishers, Inc.*, New York, pp.452-453.
- Scheibmeir, H.D., Christensen, K., Whitaker, H.S., Jegaethesan, J., Clancy, R. and Pierce, D. J. (2005). A review of free radicals and antioxidants for critical care nurses. *Intensive and Critical Care Nursing*, **21**:24-28.
- Stahl, W., Heinrich, U., Jungmann, H., Sies, H., and Tronnier (2000). Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. *American Journal Clinical Nutrition*, **71**:795-798.
- Stahl, W. and Sies, H. (2003). Antioxidant activity of carotenoids. *Mol Aspects of Med.*, **24**:345-351.
- Suman, S., Madan, K. and Seema, T. (2012). Application of apple as ripening agent for banana. *Indian Journal of Natural Products and Resources*, **3**(1):61-64.
- Sulaiman, S., Ibrahim, D., Kassim, J., and Sheh-Hong L., (2011). Antimicrobial and antioxidant activities of condensed tannin from *Rhizophora apiculata* barks. *Journal of chemistry and pharmaceutical research*, **3**(4):436-444.
- Van-Burden, T.P., and Robinson, W.C. (1981). Formation of complexes between protein and tannin acid. *Journal of Agricultural and Food Chemistry*, **1**: 77-82.
- Wang, S.Y. and Lin, H.S. (2000). Antioxidant activity in fruit and leaves of blackberry, raspberry and strawberry varies with cultivar and development stage. *Journal of Agricultural and Food Chemistry*, **48**:140-146.
- Wayne, W. F., Veazie, P.P. and Julie, K.C. (2002). Quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, **15**:309-317.
- Zakaria, H., Simpson, K., Brown, P.R. and Krotulovic, A. (1979). Use of reversed phase HPLC analysis for the determination of provitamin A, carotenes in tomatoes. *Journal of Chromatography*, **176**:109-317.