EFFECT OF RIPENING AGENTS ON THE NUTRITIONAL AND ANTIOXIDANT QUALITIES OF DIFFERENT BANANA CULTIVARS

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Abstract
Changes in chemical composition and antioxidant activities naturally occur in fruits during the process of maturation and ripening. This natural process of ripening is often stimulated using ripening agents. In this study, changes in the nutritional contents of three species of banana: Musa dwarf Cavendish (MDC), Musa sapientum linn (MSL) and Musa acuminata red daca (MARD), ripened with calcium carbide (CaC_2) and red apple were investigated. Proximate, mineral and anti-nutrientcompositions were determined using the method of A.O.A.C and antioxidant activities were quantified by UV spectrometry. $CaC_2$and red apple were found to be very effective inpeel colour development with short ripening time compared to naturally ripened banana.The three cultivars show moisture content between 71.30% and 73.33%, with CaC_2 induced bananas having the highest moisture but lowest protein content (1.40% to 1.70%) across the three cultivars. Protein content of apple induced banana was highest in MSL (1.73%) and MDC (1.57%) compared to others.Protein, carbohydrate and moisture contents as well as catalase and superoxide dismutase (SOD) activities were significantly reduced across the cultivars in CaC_2 treated banana compared with the control. Red apple significantly reduced glutathione peroxidase activity in the three cultivars but compared well with the control in protein and moisture contents as well as SOD and catalase activities. Fe contenst ofCaC_2 induced cultivars were found to be significantly high ranging from 35-83ppm.
The study concludes that CaC_2hastens ripening but reduces nutrient composition of banana while red apple is a safe alternative for fruit ripening.

Key words: ripening; *Musa dwarf Cavendish;Musa sapientum linn* and *Musa acuminata red daca*; calcium carbide; red apple and nutrient content.

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1. INTRODUCTION

Banana is the fourth most important food crop in the World (Agric Statistics at a glance, 2009) and one of the most consumed fruits in tropical and subtropical regions (Alkarkhi *et al*., 2010). About 35 different species of banana in the genus Musa have been identified. The genus Musa includes several species and cultivars out of which *Musa cavendish, Musa paradisiaca, Musa nana* and *Musa sapientum* are widely cultivated in south-western Nigeria. *Musa sapientum* is cultivated primarily for its fruits and to a lesser extent fiber production (Anhwange et al., 2009). The Red banana is a triploid cultivar of the wild banana *Musa acuminata*, belonging to the Cavendish group (AAA) which originated from Vietnam and China, but are sold throughout the World (Michel *et al*., 2002).

Bananas are an important component of a healthy diet and it is available all year round and cheap compared to other fruits like apple, mango etc. Banana is one of the anti-oxidative foods (Kanazawa and Sakakibara et al., 2000) and a powerful secondary antioxidant source (Haripyaree *et.al,* 2010). It is extremely nutritious (Sharrock and Lustry, 2000) and its consumption rate is high due to its low price.

Ripening is the final stage of development of a fruit which involves series of physiological and biochemical events leading to changes in colour, flavour, aroma and texture that make the fruits both attractive and tasty. The ripening process introduces numerous qualities and nutritional characteristics to fruits (Payasi and Sanwal 2005). In general, a fruit becomes more

edible, sweeter, less green and softer as it ripens (Rahman *et.al* 2008). Ethylene (C_2H_2) produced and released by rapidly-growing plant tissues is the chief regulator of the ripening process. Climacteric fruits like banana are often harvested in a mature but unripe condition and then subsequently allowed to ripen naturally. However, natural ripening in some fruits is a slow process, which results in high weight loss, fruits desiccation, uneven ripening and subsequently economic loss (Umesh et al 2016). Thusartificial ripening of fruits for commercial purposes is employed and this is achieved by utilizing different synthetic chemicals such as ethylene gas, ethephon, ethylene glycol, etherel, kerosene and calcium carbide (Singal et al, 2012); or natural agents likeAfrican bush mango fruit (*irvingia gabonesis*), Palm nut, Cassia leaves, Yellow Pawpaw leaves etc. (Ajayi and Mbah, 2007).
The effect ofartificial ripening agents on the nutritional value of food and human health has been a national and global concern.Lee and Kader, (2000)reported that the antioxidant ability of many fruits including banana depends on the cultivar, maturity and ripening stage, although the effects of ripening agents on some banana cultivars is relatively known, as far as we know no work was developed on how ripening agents affects antioxidants activities of banana cultivars. Thus it is imperative to study the effects of these agents on the nutrient and antioxidant composition of banana being a widely cultivated and commonly consumed food crop.

This study was therefore, designed to determine the effect of a ripening agent onnutritional and antioxidant enzymes of three banana cultivar.

2. MATERIALS AND METHODS

Sample Materials: Matured unripe banana samples and ripe red apple purchased from Bodija market in Ibadan, Oyo State were transported to theNutritional Biochemistry Unit, Department of Biochemistry, University of Ibadan, Nigeria. The maturity stage and colour of the banana samples were standardized by visual measurement and then authenticated at the Botany and Agronomy Department, University of Ibadan, Nigeria to be *Musa sapientium linn, Musa acuminata red daca* and*Musa dwarf cavendish.* All reagents used are of analytical grade. All tests were carried out at room temperature and in triplicates.

Experimental grouping: The banana fruits were separated from the bunch and divided into three treatment groups. Each group is made of 15 banana fruits (5 fruits from each cultivar) in a wooden basket under the same atmospheric condition but with different ripening agents.
Control group: This is the untreated group (control) in which bananas were allowed to ripen naturally without ripening agent.
Calcium Carbide treated group: Banana samples in this group weretreated with calcium carbide according to the method described by Sarananda (1990).

Red Apple treated group: The bananasamples in this group were ripened using red apple at a ratio of 1:5 i.e 5 banana samples to 1 apple as described by Gandhi et al., 2016
The changes in colour, if any, in extent of ripening were observed regularly and recordeddaily during the experiment. The experiment was terminated on the tenth day.

Physical analyses: The skin colour, texture and general acceptability of the samples were evaluated for both tests and control. The peel colour was monitored at C1-7 stage using the numerical colour index developed by the United fruit sales corporation (1975).
Sensory evaluation: A panel of 10 was randomly selected to determine the organoleptic attributes of the ripened banana varieties.

Proximate analyses:Proximate analyses were carried out on samples using the AOAC (1990).
Moisture content: Moisture content was estimated by gravimetric measurement of weight loss after drying 5g of banana sample in a thermostatically controlled oven at 105°C until constant weight was obtained. The moisture content of each banana variety was calculated as loss in weight of the original sample and expressed as percentage moisture content.
Ash content Ash content was obtained by heating 2 g each of the banana samples in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. It was then cooled in a desiccator and weighed at room temperature to get the weight of the ash.
***Determination of crude fibre content*:**Five(5) g each of the banana samples and 200 ml of 1.25 % H_2SO_4 were heated for 30 min and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid free. Then 200 ml of 1.25% NaOH was used to boil the residue for 30 minutes, it was filtered and washed several times with distilled water until it was alkaline free. It was then rinsed once with 10% HCl and twice with ethanol. Finally it was rinsed with petroleum ether three times. The residue was put in a crucible and dried at 105^{o} C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550^{o} C for 90 minutes to obtain the weight of the ash.
***Protein determination*:**This was determined by Kjeldahl method. The nitrogen content was analysed by digesting the samples in hot concentrated hydrogen tetraoxosulphate (VI) acid, distilled in an alkaline medium into boric acid and finally titrated with standard hydrochloric acid. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C., 1990).
***Estimation of crude lipid content*:**This was performed using the Soxhlet extraction method. To extract the lipid, 200 ml of n – Hexane was used.
***Determination of carbohydrate content*:**The carbohydrate content was determined by subtracting the summed up percentage compositions of moisture, protein, lipid, fibre, and ash contents from 100 (Otitoju, 2009).

Mineral analyses:The method of A.O.A.C (1990) was employed for the determination of mineral content. 2g each of the samples was placed in a crucible and ignited in a muffle furnace at 550^{o}C for 6 hours. The resulting ash was dissolved in 10 ml of 10 % HNO_3 and heated slowly for 20 minutes. After heating, it was filtered and the filtrate was used for the determination of mineral content. Atomic absorption spectrophotometer (AAS) was used to determine Magnesium, Iron and Phosphorus.
Antioxidant enzyme extraction and activity assay
Enzyme extraction: This was carried out on fresh ripe banana samples using the method of Yang *et al*. (2009): 1g each of the samples was weighed and homogenized with 4ml of appropriate buffer and 6ml of EDTA. The homogenate was centrifuged at 12,000rpm for 16minutes. The supernatant was removed and pellet discarded. The supernatant was kept for further analysis and antioxidant enzyme activities were estimated.
Glutathione peroxidise (GPX)assay: GPX activity was measured using fortress diagnostic

kit as reported by Olaiya et al 2017. Necessary preparation was done following the procedure in the kit. The 2.65mls of the reaction mixture contained 0.05ml of distilled water, 2.5ml of working reagent (GPx reagent provided R2) and 0.1ml dilute cumene hydroperoxide. Absorbance was read at 340nm with the initial reading taken and subsequently at 1 and 2 minutes.

Catalase assay: Catalase activity was determined according to method described by Aebi and Luck (1984). The decomposition of H_2O_2 was followed as a decrease in absorbance at 240nm in a UV spectrophotometer. The 1ml reaction mixture contains potassium phosphate buffer (pH 7.0), 250µL of enzyme extract and 60mM H_2O_2 to initiate the reaction. The reaction was measured at 240nm for 3minutes and H_2O_2 consumption was calculated using extinction coefficient, 39.4$mM^{-1}Cm^{-1}$

Superoxide dismutase (SOD) assay: SOD activity was assayed using the fortress kit method Olaiya et al 2017. The 2ml assay reaction mixture contained 50µl of diluted sample, 1.7ml mixed substrate (0.05mM/L xanthine and 0.025mM/L 2-4 iodophenyl-3-4-nitrophenol-5-phenyl tetrazolin chloride (INT)) and 250µl of xanthine oxidase. The three categories of reaction mixture were incubated for 30secondas at 37^0C and absorbance was read at 505nm with the first reading taken and subsequent readings were taken after 1,2 and 3minutes.

PHYTOCHEMICAL ANALYSES:

Determination of phytate: The phytate content was determined by the method of Young and Greeve (1940). Thisis based on the ability of the standard ferric chloride to precipitate phytate present in the banana samples. Then 1g of the sample was homogenized in 25 ml of 0.5 M HNO_3 and centrifuged at 4,000rpm for 10 min. 1 ml of 0.03 M ferric solution was added to the supernatant and left to stand for 15 min in order to allow chelation of the iron molecules by the indigenous phytate. At the end of the incubation, it was capped and heated for 20 min, 7.5 ml of distilled water was added to it. Thereafter, 0.1 ml of 1.33 M NH_4SCN (Ammonium sulphocyanide) solution was added and absorbance read at 465nm. The amount of phytate was extrapolated from a standard calibration curve for calcium phytate.

***Determination of oxalate*:** The titrimetric method of Day and Underwood (1986) was used. 150 ml of 15N H_2SO_4 was added to 5g of the sample and the solution was carefully stirred intermittently with a magnetic stirrer for 30 minutes and filtered using Whatman No 1 filter paper, after which 25 ml of the filtrate was collected and titrated against 0.1 N $KMnO_4$ solution until a faint pink color appeared that persisted for 30 seconds.

***Determination of saponin*:** Saponin composition was determined using the gravimetric method of Hudson & El-Difrawi (1979). 220ml of 20% ethanol was added to 10 g of the blended banana samples and stirred using a magnetic stirrer for 12 hours at 55° C. The solution was filtered using Whatman No 1 filter paper and the extract was reduced to 40 ml under vacuum and 20 ml Diethyl ether was added in a separating funnel and shaken vigorously. The ether layer was discarded while the pH of the aqueous solution was adjusted to 4.5 by adding NaOH. 60 ml of n-butanol was finally used for extraction. The Butanol extract were washed twice with 10ml of 5 % NaCl and evaporated to dryness in a fume cupboard to give a crude saponin which was weighed.

***Determination of tannin*:** Tannin was determined by the spectrophotometric method of Trease and Evans (1989). 5g of the banana samples were extracted with 20ml of warm water and filtered. Then 0.5ml of the filtrate was added to 0.5 ml of 0.5M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color development. The absorbance was read at 760 nm and the amount of tannin was extrapolated from a standard calibration curve for tannic acid.

Total carotenoid:Carotenoid was determined by the method of Lichtentaler and Wellburn 1983. 1g of banana sample was weighed and homogenized in 10ml of 100% acetone. The homogenate was centrifuged at 4000 rpm for 5minutes. The supernatant was separated and

absorbance read at 452nm and the amount of total carotene was calculated

Flavonoid: Total flavonoid content was determined by the method of Olivera (2008) with modifications. 500 µL of extracted sample were added 2.0 ml distilled water and 150 µl 5% $NaNO_2$ and left to incubate for 5 min. After that was added 150 µl 10% $AlCl_3$ and incubate for 6 min. Then 1ml 1M NaOH and 1.2 ml distilled water were added. Solution was mixed and incubated at 18ºC in dark for 20 min. Absorbance was measured at 510nm using Spectrophotometer. A standard curve was constructed based on a range of catechin concentrations (400 mg L^{-1})

STATISTICAL ANALYSIS: Results were reported as Mean ± Standard Error of Mean (SEM). Analysis of variance was used to determine the statistical differences among the means and results were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

***Physical and sensory analyses result*:**

Assessment of the physical features of the ripened banana showed that CaC_2 and red apple treated banana have general acceptability due to the bright peel colour. However, CaC_2 induced banana was adjudged to have a starchy (unripe) taste.This may be that it only enhanced peel colour development but did not concomitantly ripen the fruit. Red apple ripened banana was adjudged to have the best colour, taste and generally acceptable compared to untreated and calcium carbide ripened banana. Thisagrees with the reports of Gandhi (2016), Peret al. (2007); Siddiqui and Dhua 2010 which state that artificially ripened fruits are nice looking from the outside but the inside remains green, raw and tasteless.Gandhi et al (2016) and Abdullahi (2018) also reported that banana ripened with apple provide product with higher nutritional and organoleptic qualities.

Peel colour development: peel colour change was first observed in the CaC_2treated banana on the 2nd day (C1 stage) in all the cultivars, which later became fresh and brightly coloured on the 5th day (C7 stage). This was closely followed by red apple treated banana which developed traces of yellow colour on the 3rd day (C1) with bright yellow colour on the 5th day (C7). Obvious colour development was not seen in the control until the 5th day (C1) and it later attained an appreciable level of ripenessafter the 7th day but not as brightly coloured as treated samples. CaC_2has been reported to induce ripening of banana within 24hours and thus commonly used by banana marketers (Ajayi and Mbah, 2007).Therefore, it can be inferred that $CaC_2$is very effective in fruit ripening as it initiated early peel colour development, followed closely by red apple.There were no significant differences in the response of the three cultivars to ripening agents. Peelcolour development which indicates the ripening time for natural and artificially ripened *Musa dwarf cavendish (MDC), Musa sapientum linn (MSL) and Musa acuminata red daca* (MARD) are represented in figure 1 below.

Proximate Analyses Result: Table 1 show a comparison of the ash, protein, fat, moisture, fiber and carbohydrate contents of naturally and artificially ripened banana samples. The three banana cultivars show a high moisture content ranging between 71.30% and 73.33% with CaC_2 induced bananas having the highest moisture content but the lowest protein content (1.40% to 1.70%) across the three cultivars. However, protein content of apple induced banana was highest in MSL (1.73%) and MDC (1.57%).These agree with the findings of Sogo-Temi et al (2014) who reported a decrease in protein content and an increase in moisture of CaC_2 induced banana but an increase in protein content of banana induced with biological agent (African mango fruit). A good amount of carbohydrates with values between 20.73% and 22.37% was also recorded with CaC_2having the least carbohydrate content across the cultivars. Ash, protein, fat and crude fiber contents were also affected in different proportions by the ripening agents when compared with the

control, however red apple induced banana compared well with the control as presented below.

Anti-oxidant enzyme activity result: Table 2 gives the Antioxidant composition of *Musa dwarf cavendish*, *Musa sapientum linn* and *Musa acuminata red daca*with the different ripening agents.The result showed significant reduction in Glutathione peroxidase activity in induced banana samples with red apple having the least value across the three cultivars whereas there were no significant difference in superoxide dismutase and catalase activities of induced MARD and MDC.Treated banana samples had the highest flavonoid contents compared with the control. Himani (2017) also reported a decrease in the nutritive and antioxidant components of induced mangoes.

Mineral composition:The Effect of $CaC_2$and red apple on mineral contents of banana is presented in figures 2-4 below. CaC_2 treated banana showed the highest iron and phosphorus content across the cultivars. Oguntade et al., (2019) also reported the highest iron content in calcium carbide induced *musa paradisiacal* with the control having the lowest iron content while Taposhe et al. (2017) reported a decrease in iron content with induced banana samples. CaC_2decreased Mg^{2+}content significantly from 780±0.10ppm to 480±0.00ppm in MSL, 570±1.00ppm to 540±0.23ppm in MDCbut increased $Mg^{2+}$from 500±1.00 to 640±0.01 in MARD. The decrease in magnesium content in red apple induced banana was not significant compared with the control.

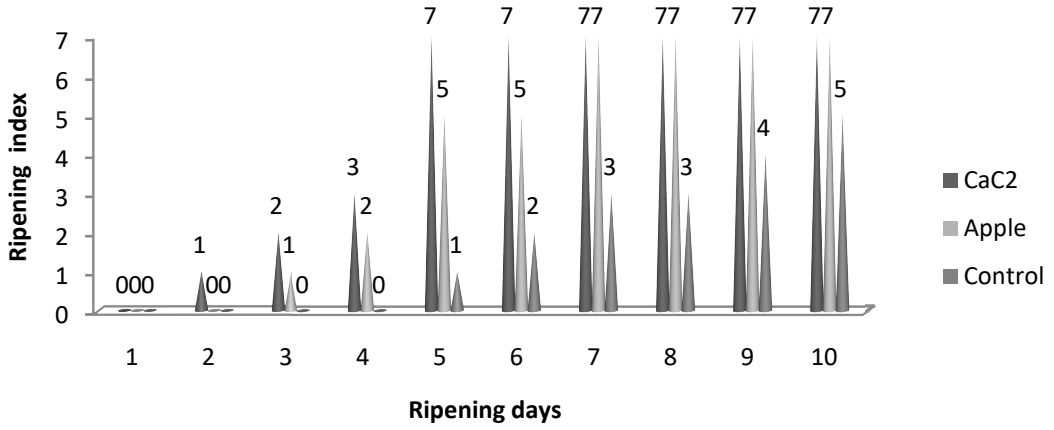

Fig. 1: Ripening index of banana with days

TABLE 1: Effect ofripening agents on proximate composition (%) ofMSL, MARD and MDC.

Sample	Ripening agents	ASH content	Total Protein	Total Fat	Moisture Content	Crude Fiber	CHO Content
MSL	Control	2.97±0.06	1.63±0.06	0.27±0.58	73.33±0.15	1.07±0.56	20.73±0.21*
	CaC_2	2.63±0.15*	1.57±0.57*	0.23±0.58	73.97±0.15*	1.03±0.58	20.57±0.31*
	Apple	2.83±0.06*	1.73±0.09*	0.20±0.10	72.14±0.65*	1.00±0.00	22.10±0.20*
MARD	Control	3.00±0.10	1.9±0.06	0.37±0.06	71.57±0.15	1.53±0.06	21.67±0.21
	CaC_2	3.23±0.15	1.70±0.10*	0.37±0.06	73.27±0.15*	1.33±0.58*	21.10±0.10*
	Apple	2.93±0.06	1.77±0.06*	0.30±0.00	71.30±0.10*	1.40±0.10	22.30±0.00*
MDC	Control	2.07±0.12	1.43±0.06	0.53±0.06	72.27±0.06	1.33±0.06	22.37±0.15
	CaC_2	2.07±0.07	1.40±0.10	0.70±0.10	73.20±0.10*	1.20±0.10*	20.53±0.15
	Apple	2.00±0.10	1.57±0.058	0.47±0.06	73.07±0.06*	1.20±0.10*	21.90±0.00*

*Values are given as means ± standard error. *represents significant differences from the control across each column@ P<0.05*

Table 2: ***Anti-oxidant activity result for Musa dwarf cavendish, Musa sapientum linn and Musa acuminata red daca*** **with the different ripening agents.**

		Enzymes				
		Glutathione peroxidise (AU $mg^{-1}min^{-1}$ Protein)	**Superoxide dismutase** (AU $mg^{-1}min^{-1}$ Protein)	**Catalase**(AU $mg^{-1}min^{-1}$ Protein)	**Flavonoid (%)**	**Total carotenoid (µg/g)**
MSL	Control	58.87±0.76	1.68±0.12	35.22±0.02	0.64±0.04	4.63±0.03
	CaC_2	25.13±0.12*	1.55±0.10*	20.57±0.40*	3.61±0.04*	9.43±0.06*
	Red apple	8.41±0.09*	1.85±0.06*	28.08±0.07*	4.41±0.04*	4.75±0.13*
MARD	Control	58.87±0.76	1.94±0.05	57.60±0.60	1.31±0.02	21.82±0.04
	CaC_2	25.13±0.11*	1.94±0.05	57.53±0.06	2.39±0.12	6.06±0.04*
	Red apple	8.41±0.10*	1.94±0.02	57.64±0.66	6.08±0.10	3.53±0.04*
MDC	Control	67.30±0.30	1.97±0.10	57.41±0.36	0.84±0.04	3.71±0.21
	CaC_2	28.41±0.41*	1.97±0.05	57.61±0.22	1.63±0.30	6.58±0.05
	Red apple	16.82±0.83*	1.98±0.07	57.68±0.72	1.55±0.07	6.56±0.52

*Values are given as means ± standard error. *represents significant differences from the control across eachcolumn @ P<0.05*

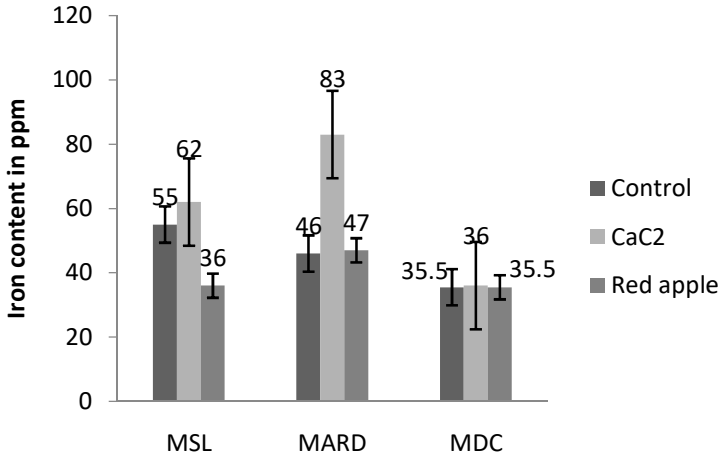

Fig. 2: The effect of ripening agents on iron content of banana cultivars

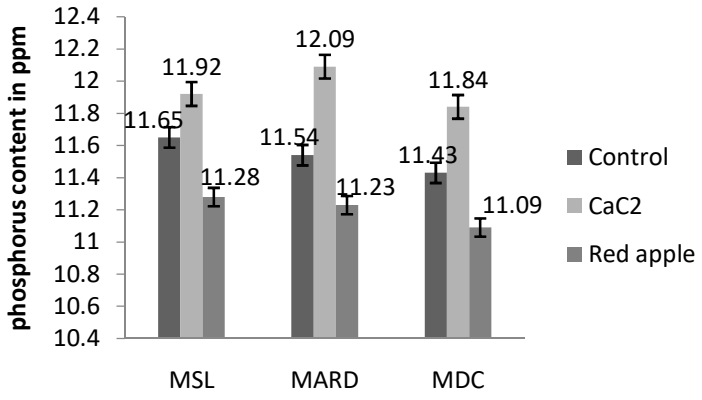

Fig. 3: The effect of ripening agents on Phosphorus content of banana cultivars

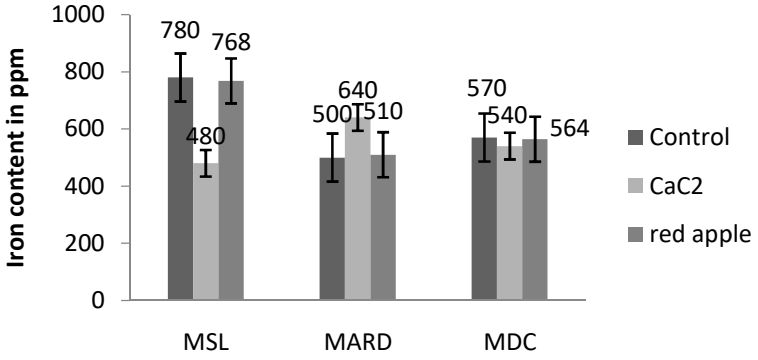

Fig. 4: The effect of ripening agents on Magnesium content of banana cultivars

Table 3: Anti-nutritional content(mg/100g) of *Musa dwarf cavendish, Musa sapientum linn and Musa acuminata red daca* with the different ripening agents.

SAMPLE	RIPENING AGENT	ANTI-NUTRIENTS			
		Saponin	Tannin	Oxalate	Phytate
MSL	Control	44.00±1.73	22.50±0.00	21.17±0.58	50.00±0.00
	CaC_2	52.67±0.58*	18.00±1.00*	18.50±0.00*	46.00±0.00
	Red apple	46.33±1.53*	18.00±2.00*	18.17±0.29*	48.00±0.00
MARD	Control	40.67±1.15	24.33±0.29*	32.77±0.15	53.67±1.53
	CaC_2	47.67±1.15*	24.83±0.76*	32.63±0.15	51.33±1.15
	Red apple	38.67±0.58*	23.50±0.00*	30.77±0.15	53.00±1.00
MDC	Control	53.33±2.89	21.67±0.76	21.40±0.17	47.33±1.15
	CaC_2	64.67±1.53*	12.50±0.57	19.83±0.29	41.67±1.53
	Red apple	51.67±0.58*	45.33±0.58	19.37±0.32	45.33±0.58

*Values are given as means ± standard error. *represents significant differences from the controlacross eachcolumn@ P<0.05*

Anti-nutrient Composition: Table 3summarizes the results of the anti-nutritional contents of the three banana cultivars. There were no significant differences in the phytate content across the cultivars, however CaC_2 showed the highest Saponin content which is significant only in MSL and MDC. Ripening agent reduced Oxalate content in all the cultivars but was only significant in MSL.

4. CONCLUSION

Although it is well known that 'one rotten apple spoils the whole bushel', the same attribute can be of immense importance when applied technically. The use of red apple in ripening fruit is not a well known phenomenon but it has proved to be economical and nutritionally adequate. Red apple in addition to causing a bright peel colour development of the banana samples also maintained and in some cases increased the nutrients in banana. Thus apple can be exploited as a natural, safe and faster ripening agent to facilitate ripening of fruits. Also, it can be concluded that different ripening agents affect banana cultivars differently as there were significant differences in the response of the three banana cultivars examined to the different ripening agents.

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