

POST HARVEST TREATMENT OF BANANA WITH ARTIFICIAL RIPENING AGENT IMPARTS ON ITS NUTRITIONAL QUALITY AND ANTIOXIDANT POTENTIAL

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Abstract: Bananas are widely consumed fruits that are often subjected to artificial ripening to accelerate their marketability for economic gains. Calcium carbide (CaC_2) is most commonly used as a ripening agent, particularly in developing countries, due to its low cost and effectiveness. However, the use of CaC_2 has raised concerns about its potential impact on the nutritional quality and safety of the treated fruits. Assessment of the impact of this practice on the nutritional value of banana is key to improving its functionality and maximize its nutriture. This study was undertaken to examine the effect of post-harvest treatment of banana with calcium carbide agent on nutrient composition and antioxidant enzymes of the pulp. Mature green bananas were separated into fingers of the same size and divided into 4 groups. Group I was untreated and unripen while groups II and III were CaC_2 -treated at 4g/kg and 8g/kg of fruit respectively. Group IV underwent natural ripening. Proximate composition, sensory evaluation and antioxidant enzyme analyses were carried on the samples after the treatment period following standard procedures. The results show that calcium carbide used at 4g/kg and 8g/kg tilted sensory properties of banana pulp towards low values but not significantly ($p > 0.05$). On the other hand, there was significant difference ($p < 0.05$) in the proximate composition of the artificially ripened banana compared to control bananas. Similarly, the levels of essential minerals decreased significantly ($p < 0.05$)—treated groups II and III compared with group 4. Furthermore, the results of antioxidant enzyme activities indicated that calcium carbide treatment compromised the levels of all the antioxidant enzymes analysed as their concentrations significantly decreased ($P < 0.05$) from 3.42 to 1.65 U/mL; 32.79 to 22.73 U/mL and 0.68 to 0.28 U/mL for peroxidase, ascorbate peroxidase and catalase respectively. These findings have shown how the nutritional composition as well as antioxidant potential of banana could be compromised if the use of chemical ripening agents such as calcium carbide continues unabated. **Keywords:** Banana pulp, artificial ripening, calcium carbide, micronutrient, antioxidant enzyme, food safety.

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Introduction

Bananas (*Musa spp.*) are one of the most widely consumed fruits in the world, with global production reaching over 117 million tons in 2019 (FAO, 2021). As important source of nutrients, including carbohydrates, fiber, vitamins (particularly vitamin B6 and vitamin C), minerals (especially potassium),—and various bioactive compounds such as phenolics, carotenoids, and biogenic amines (Singh *et al.*, 2016; Sidhu & Zafar, 2018), it is considered a popular staple 'food' for more than 400 million people globally (Sanjeev and Eswaran, 2008). Bananas are typically harvested at the mature green stage [along with judging the angularity of fruit \(Prasad et al. 2018a\)](#) and then ripened artificially to achieve the desired peel color, texture, and flavor attributes demanded by consumers (Maduwanthi & Marapana, 2019). The need to

Comment [KP4]: Please insert the following reference
Prasad, K., Siddiqui, M. W., Sharma, R. R., Gaurav, A. K., Neha, P., & Kumar, N. (2018a). Edible coatings and their effect on postharvest fruit quality. *Innovative Packaging of Fruits and Vegetables: Strategies for Safety and Quality Maintenance*, Apple Academic Press, pp 161-197.

meet consumers' demand driven by the desire to make profit by fruit sellers has resulted to the practice of artificial ripening with chemical agents that often impact on the physicochemical properties cum nutritional quality of fruits. Some of the most common ripening chemicals include ethylene gas, ethephon, ethylene glycol, ethereal, ~~torch-light battery~~ and potash, ash (Singal *et al.*, 2012), while natural agents inevitably used for the purpose of transporting fruits across long distances include Cassia leaves and African bush mango fruit (*#Irvingia gabonesis*) (Adewole and Duruji, 2010). However, the most common methods of artificial ripening is the use of calcium carbide (CaC_2), particularly in developing countries. When CaC_2 comes into contact with moisture, it generates acetylene gas, an analog of ethylene, which accelerates the ripening process (Asif, 2012; Siddiqui & Dhua, 2010). Although artificial ripening with CaC_2 has been effective and inexpensive, it does raises concerns due to potential health risks associated with the presence of chemical residues such as arsenic and phosphorus hydride on treated fruits (Banjare *et al.*, 2014; Hakim *et al.*, 2012 and Ruwali *et al.*, 2022). Moreover, several studies have reported that artificial ripening with CaC_2 can lead to changes in the nutritional composition and antioxidant properties of fruits. In the absence of specific regulatory frameworks, particularly in developing countries such as Nigeria to control artificial ripening, research-informed public sensitization is key towards curbing such unwholesome practices. Although, available literature ~~have~~has contributed to some awareness on the use of calcium carbide, there is however contrasting information on its impact on nutritional value and paltry report on antioxidant enzyme content. Consequently, this study was designed to further buttress the practice of using chemical agents for ripening of fruits by assessing the effect of different concentrations of calcium carbide on the nutritional quality of banana. Additionally, the activities of key antioxidant enzymes, namely POX, APX and CAT were evaluated to provide insights into the effects of CaC_2 treatment on the antioxidant defense system of banana.

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Materials and methods

Sample collection and preparation

Freshly harvested bunch of ~~mature unripe banana~~ was collected from Railway fruit market in Makurdi, Benue State Nigeria, and transported immediately to the laboratory. Using visual measurements of maturity stage and color, banana samples were standardized and authenticated at the Botany Department of Joseph Sawuan Tarka University, Makurdi to be *Musa spp.* Banana fingers were thereafter separated from the bunch and thoroughly washed under running water to

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remove dirt and other foreign materials. On the other hand, the most common ripening agent, calcium carbide was purchased from a local welding shop and prepared in two graded concentrations of 4g and 8 g before treatment as outlined in the experimental design.

Comment [KP8]: Please give authentic source of purchase

Experimental grouping and post-harvest treatment of samples

The clean banana fingers were grouped into four with each group having four fingers of approximately same size, and labeled as shown below:

Group 1: Unprocessed and untreated banana used as negative control (NGC)

Group 2: banana ripened with 4 g calcium carbide

Group 3: banana ripened with 8 g calcium carbide

Group 4: naturally ripened banana used as positive control (PTC)

Group 1 was unprocessed and untreated serving as a negative control. Groups 2 and 3 were treated with 4 g and 8 g of calcium carbide respectively, and wrapped separately in polythene before being dropped into a black polythene bag containing the bananas. The starting concentration of ripening agent was chosen based on description anonymously given by some users. Lastly, samples in the 4th group were placed in the polythene bag without ripening agent and served as positive control group. Sample bags were appropriately labeled and kept under the same experimental conditions while being monitored daily. Samples were carefully observed for changes in peel colour using ASDA Colour Scale of 1-7, with each number corresponding to a specific colour stage. Complete ripeness was determined at stage 5 for this study. Ripening time and shelf life were thereafter determined using a method described by Adeyemi *et al.*, (2010).

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Sensory evaluation

Sensory analysis was carried out using the seven-point hedonic scale (where 1 = dislike much and 7 = like much) as described by Saeed *et al.* (2001). The organoleptic properties evaluated were appearance, sweetness, aroma, firmness, mouth feel and general acceptability. The sensory panel consisted of 20 semi-trained panelists selected from postgraduate students (aged 27 - 35 years) of Center for Food Research and Technology (CEFTR), Benue State University, Makurdi.

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Proximate analysis

Proximate analysis of samples was carried out according to approved standard procedures of Association of Official Analytical Chemist (AOAC, 2019). In summary, moisture content was

determined in a hot-air circulating oven (Gallenkamp, UK) where 5.0 g of sample was weighed into a known weight of crucible placed in an oven and dried at 105 °C for 3 h (AOAC 930.15). The percentage moisture content was calculated by expressing percentage of weight relative to the initial sample weight (loss in weight on drying as a fraction of the initial weight of sample used and multiplied by 100). Crude fibre content was determined by weighing 5 g of sample and heating with 200ml of 1.25% H₂SO₄ for 30 min and filtered with a Buchner funnel. The residue was made acid-free through thorough washing before adding 200 ml 1.25% NaOH and boiled for another 30 min. Following another thorough washing to remove any trace of alkaline, it was thereafter rinsed with 10% HCl and twice with ethanol before final rinsing with petroleum ether thrice. Thereafter, the residue was put in a crucible and oven-dried at 105 °C overnight. Later, the sample was cooled in a desiccator and ignited in a muffle furnace at 550 °C for 90 min to obtain the weight of ash (AOAC 978.10). The percentage of crude fibre was then calculated through weight differentials from weight after drying/ weight of sample x 100. For total ash content, samples of known weights were incinerated at 550°C in a muffle furnace (Gallenkamp, UK) for 3 h (AOAC 942.05), and percentage ash content was calculated from weight of ash /weight of original sample x 100. Crude fat determination was done by completely extracting a known weight sample in petroleum ether for 4h (AOAC 2003.06). Percentage fat was then calculated using the formula: % fat = weight of fat/weight of sample × 100. Crude protein content was determined using the micro Kjeldahl method involving digestion and distillation (AOAC 2001.11). The percentage crude protein was calculated from the % Nitrogen as: % crude protein = % N x F, where, F (with conversion factor equivalent to 6.25. The carbohydrate content was measured by difference using the equation: % carbohydrate = 100 – (% moisture + % crude fibre + % crude protein + % crude fat + % ash).

Determination of Elemental Content

Determination of mineral content was carried out according to AOAC methods (AOAC, 2019) for the following elements; iron, calcium, magnesium, zinc, copper, manganese, and heavy metals lead and cadmium. Briefly, measured sample was acid-digested by adding 20 ml H₂SO₄ and 10 ml of HNO₃ in the ratio 2:1. The mixture was heated on a bunsen burner until the brown fumes subsided. Addition of 10 ml of HNO₃ continued at the interval of 10 minutes and heated

until the solution turned colourless, and filtered. The digested samples were then analyzed using atomic absorption spectrophotometer (AAS).

Antioxidant Enzyme Assay

Banana peels were removed and 10 g of pulp was homogenized in 50 mL of 100 mM, potassium phosphate buffer (pH 7.8) containing 1 mM ascorbic acid and 0.5 % (w/v) polyvinylpyrrolidone (PVP), 2 mM EDTA for 5 min using a pre-chilled mortar and pestle according to Pandey *et al.*, 2013. The resultant homogenate was filtered through three layers of cheese cloth. The filtrate was centrifuged at 5000 x g for 15 min using refrigerated centrifuge at 4°C. Supernatants were collected as crude extracts and used immediately in each case for the enzyme assays.

Measurement of Peroxidase (POX) Activity

Peroxidase activity was measured by performing the guaiacol oxidation method according to the Rao *et al.* (1996) with slight modifications. The reaction mixture 3 ml contained 50 mM phosphate buffer (pH 6.8) with 2.7 mM guaiacol and 50 µl pulp extract in which 4 mM H₂O₂ was added to initiate the reaction. The reaction mixture was made homogenous through incubation for 5 min. The absorbance measured at 470 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu Cooperation, Japan) was monitored for change per min for 30 min. The Activity was determined using extinction coefficient 6.39 mM⁻¹ cm⁻¹ and expressed as unit per milligram of protein.

Measurement of Ascorbate Peroxidase (APX) Activity

Ascorbate peroxidase activity was determined using the modified method described by Kumar (2021). The reaction mixture 5 ml contained 90 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 1 mM ascorbate and 1 mM H₂O₂ and 50 µl of crude extract. The APX activity was determined by following the H₂O₂ decomposition and decrease in ascorbate at 290 nm using a UV-vis spectrophotometer (UV-1800, Shimadzu Cooperation, Japan). The enzyme activity calculated using the molar extinction coefficient absorbance of molar solution) of 2.8 mM⁻¹ cm⁻¹ was expressed as unit per milligram of protein.

Measurement of Catalase (CAT) Activity

Catalase activity was determined following the protocol documented by Ali *et al.*, 2005. The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 2 mM H₂O₂ and 1mM EDTA and 50 µl of crude extract. Catalase activity was monitored by the decomposition of H₂O₂ measured at 240 nm using the extinction coefficient of 39.4 mM⁻¹ cm⁻¹ and expressed as µmol H₂O₂/min/mg protein.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD) and analyzed by analysis of variance (ANOVA) using SPSS program (version 20.0 SPSS Inc., Chicago, IL, USA) followed by Duncan's multiple range post hoc test. Significance was defined as P < 0.05.

Results

Effect of calcium carbide-induced ripening on the ripening time of banana

The result presented in Table 1 shows that bananas treated with 4 g/kg calcium carbide (GRP 2) ripened within 3 days (72 h) while the bananas treated with 8 g/kg calcium carbide (GRP 3) had a ripening time of 2 days (48 h). Naturally ripened bananas (GRP 4) took a longer time of 7 days (168 h) before ripening.

Table 1: Effect of calcium carbide-induced ripening on the ripening time of banana

| Sample | CaC ₂ (g/kg) | Ripening time (days) | Shelf life (days) |
|--------|-------------------------|----------------------|-------------------|
| Grp 1 | 0 | 0 | 0 |
| Grp 2 | 4 | 3 | 4 |
| Grp 3 | 8 | 2 | 3 |
| Grp 4 | 0 | 7 | 6 |

GRP 1 – Unprocessed and untreated banana; GRP 2 – banana ripened with 4g CaC₂; GRP 3 – banana ripened with 8g CaC₂ and GRP 4 – Naturally ripened banana.

Effect of calcium carbide-induced ripening on organoleptic properties of banana pulp

The result of sensory evaluation is shown in Table 2. There was a significant difference (p < 0.05) in the appearance of unprocessed and untreated samples (GRP 1) compared to other groups. Comparatively, GRP 3 had the best appearance score at 5.43 and also the highest acceptability at 4.43. On the other hand, sweetness scores of the samples ranged between 0.23 and 4.33 with GRP 3 noted as the sweetest. The sample aroma score was lowest in GRP 1 at 0.32 while GRP 3 had the highest aroma at 4.12. On firmness, GRP 1 samples was observed with the

highest score at 5.50, which is statistically different ($p < 0.05$) from other groups as indicated in the table. Mouth feel and acceptability were significantly higher ($p < 0.05$) in GRP 3 than the rest of the groups while the unripe banana (GRP 1) had the least scores in these two organoleptic properties.

Table 2: Effect of calcium carbide-induced ripening on sensory properties of banana pulp

| Sample | CaC ₂ (g/kg) | Appearance | Sweetness | Aroma | Firmness | Mouthfeel | Acceptability |
|--------|----------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| GRP 1 | 0 | 2.22 ± 0.32 ^a | 0.23 ± 0.18 ^a | 0.32 ± 0.15 ^a | 5.50 ± 0.22 ^c | 0.23 ± 0.14 ^a | 2.23 ± 0.25 ^a |
| GRP 2 | 4 | 5.42 ± 0.55 ^b | 2.89 ± 0.11 ^b | 3.25 ± 0.13 ^b | 3.98 ± 0.23 ^a | 2.98 ± 0.99 ^b | 3.33 ± 0.46 ^b |
| GRP 3 | 8 | 5.43 ± 0.33 ^b | 4.33 ± 0.89 ^c | 4.12 ± 0.21 ^c | 5.12 ± 0.23 ^c | 4.12 ± 0.30 ^d | 4.43 ± 0.34 ^c |
| GRP 4 | 0 | 5.40 ± 0.23 ^b | 2.97 ± 0.67 ^b | 3.21 ± 0.45 ^b | 4.43 ± 0.22 ^b | 3.22 ± 0.24 ^c | 3.23 ± 0.56 ^b |

GRP 1 – Unprocessed and untreated banana; GRP 2 – banana ripened with 4g CaC₂; GRP 3 – banana ripened with 8g CaC₂ and GRP 4 – Naturally ripened banana.

Effect of calcium carbide-induced ripening on proximate composition of banana pulp

Shown in Table 3 is the result of proximate analysis of samples. The results indicated that the moisture content of samples in Groups 1 and 2 are significantly different ($p < 0.05$) from that of groups 3 and 4 with GRP 4 having the highest value of moisture content at 77.78% while group 2 had the lowest value of 69.40%. The fat content of samples ranged between 0.65% to 0.81% with groups 4 and 1 having the highest and lowest amount respectively. The fibre content was highest in GRP 1 recorded as 3.03% while GRP 2 has the lowest amount recorded as at 1.83%. The ash content ranged between 1.59% in GRP 1 and 2.78% with this group being significantly different ($p < 0.05$) from other groups. The carbohydrate content of the samples in GRP 2 appeared highest compared to others, which ranged from 15.23 to 24.39%.

Table 3: Effects of calcium carbide-induced ripening on proximate composition of banana pulp

| Sample | Moisture % | Fat % | Ash % | Fibre % | Protein % | CHO % |
|--------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|----------------------------|
| Grp 1 | 68.40 ± 1.54 ^a | 0.65 ± 0.01 ^a | 2.48 ± 0.13 ^c | 3.03 ± 0.06 ^d | 2.22 ± 0.05 ^c | 21.89 ± 1.51 ^{bc} |
| Grp 2 | 69.79 ± 3.11 ^a | 0.68 ± 0.01 ^b | 2.03 ± 0.06 ^b | 2.06 ± 0.04 ^a | 1.67 ± 0.01 ^a | 20.39 ± 3.04 ^c |
| Grp 3 | 76.50 ± 0.70 ^b | 0.72 ± 0.04 ^c | 1.88 ± 0.03 ^a | 1.83 ± 0.03 ^b | 1.36 ± 0.09 ^b | 17.68 ± 0.71 ^{ab} |
| Grp 4 | 77.78 ± 0.80 ^b | 0.81 ± 0.06 ^d | 2.59 ± 0.09 ^d | 2.32 ± 0.08 ^c | 2.25 ± 0.13 ^c | 25.23 ± 0.92 ^a |

GRP 1 – Unprocessed and untreated banana; GRP 2 – banana ripened with 4g CaC₂; GRP 3 – banana ripened with 8g CaC₂ and GRP 4 – Naturally ripened banana.

Effect of calcium carbide-induced ripening on mineral composition of banana pulp

Table 4 represents the result for the effect of CaC₂- treated samples on mineral composition in comparison with unprocessed and naturally-ripened banana pulp. The results show that GRP 4, which were allowed to ripen naturally had the highest amount of micronutrients Zn and Mg at 6.08 and 9.12 ppm respectively, and are significantly higher than those of the CaC₂-induced ripened samples of groups 2 and 3. There was a decrease in Pb content during ripening. The naturally ripened bananas with no ripening agent (GRP 4) and GRP 2 had the lowest Pb value at 0.02 ppm, while GRP 1 (unripe banana) had significantly higher ($p < 0.05$) value of 0.07 ppm. There were significant ($p < 0.05$) difference in the Mn, Ca and Cd contents (2.42 ppm, 2.24 ppm and 1.46 ppm respectively) in the banana treated with 8 g/kg CaC₂ (GRP 3) when compared with the rest of the groups. Cu content ranged from 0.38 in GRP 4 to 1.64 in GRP 1.

Table 4: Effect of calcium carbide-induced ripening on mineral composition of bananapulp

| Sample | Lead (ppm) | Copper (ppm) | Zinc (ppm) | Manganese (ppm) | Magnesium (ppm) | Calcium (ppm) | Iron (ppm) | Cadmium (ppm) |
|--------|------------------------|------------------------|------------------------|------------------------|-------------------------|------------------------|---------------|------------------------|
| Grp 1 | 0.07±0.01 ^b | 1.64±0.09 ^c | 5.29±0.85 ^b | 0.20±0.05 ^a | 8.92± 0.52 ^a | 0.01±0.00 ^a | ND | 1.13±0.02 ^a |
| Grp 2 | 0.02±0.01 ^a | 0.49±0.22 ^b | 2.19±0.18 ^a | 0.39±0.12 ^a | 6.06± 1.03 ^b | 1.42±0.48 ^b | ND | 1.20±0.01 ^a |
| Grp 3 | 0.03±0.01 ^a | 0.50±0.03 ^b | 1.99±0.48 ^a | 2.42±0.12 ^b | 5.49± 0.21 ^c | 2.24±0.20 ^c | ND | 1.46±0.05 ^b |
| Grp 4 | 0.02±0.01 ^a | 0.78±0.01 ^a | 6.08±0.08 ^b | 0.14±0.03 ^a | 9.12± 0.37 ^a | 0.01±0.00 ^a | ND | 0.98±0.01 ^a |

GRP 1 – Unprocessed and untreated banana; GRP 2 – banana ripened with 4g CaC₂; GRP 3 – banana ripened with 8g CaC₂ and GRP 4 – Naturally ripened banana. ND – Not detectable

Effect of calcium carbide-induced ripening on antioxidant enzyme activity of banana pulp

Table 5 shows the effect of calcium carbide treatment of banana samples on some antioxidant enzymes (peroxidase, ascorbate peroxidase and catalase). The results show that peroxidase activity was significantly higher ($p < 0.05$) in the naturally-ripened banana of GRP 4 at 3.42 U/mL than the artificially-ripened samples in groups 2 and 3. Similarly, the amounts of ascorbate peroxidase and catalase in CaC₂-treated samples of groups 2 and 3 are significantly lower than their counterpart values in naturally-ripened banana in group 4.

Table 5: Effect of calcium carbide-induced ripening on antioxidant enzyme activity of banana pulp

| Sample | POX (U/mL) | APX (U/mL) | CAT U/mL) |
|--------|--------------------------|----------------------------|--------------------------|
| Grp 1 | 1.59 ± 0.06 ^a | 28.15 ± 9.61 ^a | 0.39 ± 0.25 ^a |
| Grp 2 | 1.65 ± 0.05 ^a | 24.67 ± 10.45 ^b | 0.33 ± 0.28 ^b |
| Grp 3 | 1.99 ± 0.19 ^b | 22.73 ± 2.40 ^b | 0.28 ± 0.24 ^c |
| Grp 4 | 3.42 ± 0.05 ^c | 33.79 ± 2.15 ^c | 0.68 ± 0.15 ^d |

GRP 1 – Unprocessed and untreated banana; GRP 2 – banana ripened with 4g CaC₂; GRP 3 – banana ripened with 8g CaC₂ and GRP 4 – Naturally ripened banana.

Discussion

An unabated practice among fruit sellers is the use of calcium carbide for artificial ripening of many fruits including banana because of its ability to release acetylene gas, which is a plant hormone that stimulates ripening. Although this chemical quickens the ripening process, the concomitant impact on the nutritional quality has been downplayed, thus further justified the rationale for this work. A key observation was that although calcium carbide ripened banana faster, it also resulted in shorter shelf life than natural-ripened bananas, which can result in increased food waste and economic losses for consumers and retailers alike. These results are in congruent with previous reports documented by Adeyemi *et al.*, (2018), who examined the effects of biological and chemical ripening agents on the nutritional and metal composition of some fruits; Sogo-Temi *et al.* (2014) who showed faster deterioration of calcium carbide-induced ripening of bananas compared to those allowed to ripen naturally. In addition to supporting existing reports, the ripening was observed to be concentration and time-dependent, indicating greater health risk and economic loss from using higher amounts of the ripening agent. In the same vein, evaluation of sensory properties revealed higher scores for artificially-ripened bananas compared to unripe samples, which concurs with the findings of Nura *et al.* (2018) who reported that use of air tight chambers during calcium carbide ripening provide better sensory properties. This observation however contradicted the result of Gunasekara *et al.* (2015) who recorded low sensory scores and low-level nutritional qualities in banana treated with calcium carbide and ethephon. The difference may arise from a cocktail of factors such as species of banana used, degree of maturity and/or condition of growth as well as the amount of the ripening agent utilized. Notwithstanding the discrepancies in the observed organoleptic properties between the present findings and previous, acceptability score was reasonably high indicating that bananas from control process of ripening are still palatable and acceptable by consumers save for the negative impacts associated with consuming such food products.

A consensus in documented reports of artificial ripening of fruits is its ability to compromise nutritional quality of such food products. Our findings show that this process greatly impacted proximate composition as well as mineral components of banana pulp in which calcium carbide-induced ripening resulted in reduced carbohydrate, protein ash and crude fibre contents compared to naturally processed bananas. These results agree with Oko *et al.*, (2018) and Olubiyo *et al.*, 2022 who observed a reduction in protein content during ripening and attributed same to possible reduction of nitrogen during ripening. In addition, Bananas generally contain small amounts of proteins such as antioxidant proteins, which may be made available through normal protein synthesis. The observed variation in amounts between artificial and naturally ripened bananas may result from a possible disruption of protein synthetic machinery by the ripening agent or its constituents. This study recorded similar values for moisture content with those reported by Nuhu *et al.*, (2020), who noted that moisture content in banana pulp is observed to increase because of respiratory breakdown of starch to sugar, migration of water from peel to pulp and excess moisture formation. The high moisture content of banana invariably contributes to its short storage life and high post-harvest loss, thus, facilitated ripening can comprise shelf life occasioned by observed high moisture content (Prasad *et al.* 2018b). The reduction in

Comment [KP11]: Please insert the following literature
Prasad, K., Jacob, S. & Siddiqui, M. W. (2018b). Fruit maturity, harvesting, and quality standards. In *Preharvest Modulation of Postharvest Fruit and Vegetable Quality* (pp. 41-69). Academic Press, USA.
<https://doi.org/10.1016/B978-0-12-809807-3.00002-0>

carbohydrate content in the present study agrees with many others but disagrees with the report of Gbarakoro *et al.* (2020), who found increase in carbohydrate content of bananas during ripening. This contrast may be ~~as a result of~~because of the higher concentrations of calcium carbide used. Theoretically, starch degradation catalysed by α and β amylases, which convert starch to simple sugars may be a plausible explanation for the observed reduction in carbohydrate content between artificially ripened bananas and naturally ripened ones. The influence of calcium carbide on proximate composition is also ~~similar to~~like those found for artificially ripened mangoes (~~by~~ Hussain *et al.*, 2024).

Fruits including bananas are a good source of minerals required in the body for the maintenance of physiochemical processes essential to life. While minerals such as potassium, magnesium, calcium, zinc, iron and copper are considered essential, heavy metals like lead, cadmium and tin are considered contaminant with toxic effect, and evidence of their requirements and essentiality in the body is weak (Soetan *et al.*, 2010). Our findings show that naturally ripened bananas were more nutrient-dense in terms of some essential micronutrients such as zinc, but were observed to be depleted in samples treated with artificial ripening agent. This observation ~~is in agreement~~agrees with other studies (Yildizlez *et al.*, 2018; Hafsa *et al.*, 2019; Fasanya *et al.*, 2020; Sojini *et al.*, 2021 and Ubuoh *et al.*, 2022) who found lower levels of micronutrients in banana exposed to chemical ripening agents. Given the increasing cases of micronutrient deficiency, practices such as chemically-induced ripening that compromises the amount of essential nutrients poses a risk to public health.

Bananas, like many other fruits ~~and vegetables~~, contain various antioxidant enzymes that play a crucial role in their nutritional value and potential health benefits. Catalase and peroxidase are one of the most important antioxidant enzymes involved in scavenging active oxygen species in plants (Huang *et al.*, 2007). Catalase which is particularly abundant in the peel of bananas is responsible for converting hydrogen peroxide, a by-product of the superoxide dismutase reaction into water and oxygen. Peroxidase enzymes are involved in the removal of various peroxides and free radicals, contributing to the overall antioxidant defence system in bananas. Although most studies in this regard concentrated on either antioxidant potential of peels or capacity pulp, our enzyme activity results in the present study revealed that calcium carbide-induced ripening reduced the activity of the antioxidant enzymes tested, which agrees with findings of studies conducted by Satya *et al.*, 2014; Sijand & Nandini 2017 and Kim *et al.*, 2022. Antioxidant enzymes in bananas may work synergistically with other phytonutrients to neutralize free radicals and prevent oxidative damage to cells and biomolecules, which is linked to various chronic diseases. Depending on cultivar, ripeness stage, storage conditions, and processing methods, adequate amounts of these enzymes in banana would complement the work of cellular defence machinery, however, this important function may be diminished by the influence of chemical ripening agents such calcium carbide.

Conclusion

This study provides additional evidence in support of the impact of CaC_2 -induced ripening on the micronutrient composition and antioxidant enzymes in banana fruit. The findings suggest that artificial ripening with CaC_2 can alter the nutritional quality of bananas, potentially reducing their health benefits for consumers. The observed changes in antioxidant enzymes may also impact the fruit's ability to resist oxidative stress and maintain shelf life. Although only one banana cultivar and a select suite of micronutrients and antioxidant enzymes were used in the study, the findings of this study underscore the importance of adopting safer and more

sustainable ripening practices that prioritize food quality, nutritional integrity, and consumer health, while preventing predisposition of consumers to toxic effects chemical ripening agents such as calcium carbide. It is imperative for regulatory authorities to establish stringent guidelines and enforce strict monitoring of banana ~~ripening in particular, and~~ ripening, and fruits in general to ensure the safety and well-being of consumers. Furthermore, raising public awareness about the potential risks associated with artificially ripened fruits and promoting the consumption of naturally ripened produce is crucial for fostering a healthy and sustainable food system. Our ~~future~~ future research endeavour would explore the effects of CaC_2 on a wider range of banana cultivars and ripening stages to better understand the genotypic and developmental factors influencing the fruit's response to artificial ripening. Additionally, more comprehensive analyses of the banana metabolome and proteome could provide deeper insights into the biochemical pathways and regulatory mechanisms underpinning the observed changes in micronutrient levels and antioxidant enzymes, which we would also explore.

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