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Evaluation of the Effect of Calcium Carbide as a Ripening Agent on the Nutritional Value and Heavy Metal Content of Banana and Orange

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Abstract
This study was carried out to ascertain the effect of Calcium Carbide on the nutritional value and heavy metal content of banana and orange. The parameters were determined using standard Association of Official Analytical Chemist (AOAC) methods. The result showed significant differences ($p < 0.05$) between the fruits ripened with calcium carbide and the untreated fruits. Untreated fruits contained more fat (0.15, 1.29 %), protein (0.88, 4.9 %) vitamin A (565.33, 282.74 µg/100g), vitamin C (102.07, 43.47 mg/100g), sucrose (42.99, 77.28 %), sodium (50.30, 70.70 ppm) and calcium (111.10, 168 ppm) than the carbide-ripened fruits (fat: 0.08, 1.02 %; protein: 0.29, 2.24 %; vitamin A: 363.10, 142.86 µg/100g; vitamin C: 73.92, 25.07 mg/100g; sucrose: 36.95, 55.15 %; Sodium: 39.55, 59.10ppm and Calcium: 97.35, 155.05 ppm) with Calcium carbide for both orange and banana samples respectively. The moisture (85.25, 44.46 %), ash (0.25, 0.63 %) and fibre content (0.52, 0.09 %) were found to be higher in the carbide-ripened fruits than the untreated fruits (moisture: 84.71, 46.10 %; ash: 0.18, 0.45 %; and fibre: 0.49, 0.07 %) for orange and banana respectively. Meanwhile, carbohydrate had higher concentration in untreated orange fruits (13.61%) than in carbide-ripened orange fruit samples (13.58%), but lower concentration in untreated banana fruit (48.75%) than in carbide-ripened banana fruit samples (50.43 %). The calcium ripened fruits (orange and banana) were found to contain traces of heavy metals (Pb and As) which was not present in the untreated fruits. The study revealed that the usage of Calcium carbide as a ripening agent caused significant reduction in the fruits nutrients and as well introduced heavy metals in them.

Keywords: Untreated fruits, carbide-ripened fruits, heavy metals, concentration

Introduction

The ultimate stage of fruit development is ripening; an irreversible process involving a slew of physiological, biochemical and organoleptic changes (Maduwanthi and Marapana, 2019). Fruit ripening is the consequence of a series of complex events, these events or changes are unrelated to one another. They include; seed maturation, colour, abscission, respiration rate, rate of ethylene production and tissue permeability. Similarly, organic acid changes, softening, development of wax on skin, production of flavour volatilities, protein composition and changes in the composition of carbohydrate are all observed (Maduwanthi and Marapana, 2019; Abbaset, 2021).

Fruits that are ripe have a short shelf life and are easily lost in transit (Mursalat *et al*., 2013). As a result, fruits are typically gathered while unripe, transported and artificially ripened upon arrival. In addition, fruit dealers who want to fulfill high demand and make more money collect immature fruits and use artificial ripening agents to speed up the ripening process. Although artificial ripening of fruits is a speedy procedure, compromises the nutritional quality, sensory experience and safety of the fruits (Hossain *et al*., 2015).

Calcium carbide is a frequent ripening agent. This is due to the fact that it is inexpensive and simple to obtain. However, intake of fruits ripened with Calcium carbide poses substantial hazard to the health of consumers (Rahim, 2012); arsenic and phosphorous traces have been found in it. Calcium carbide is a carcinogen that can also cause neurological problems. It can cause tingling in the hands and feet, as well as peripheral neuropathy (Rahim, 2012). Fruits ripened with Calcium carbide are exceedingly harmful to one's health,

especially the neurological system. Acetylene, which is produced from carbide, limits the amount of oxygen available to the brain. It causes headache, vertigo, disorientation, delirium, seizures and even coma in its early stages. It has the potential to cause mood swings and memory loss in the long run. Abdominal discomfort, vomiting and diarrhoea have been reported after intake of fruits ripened with Calcium carbide. Skin burns, allergies and jaundice are some of the other negative effects (Fattah and Ali, 2010).

Artificial ripening has been reported to have negative effects on the nutritive value of fruits which can reduce the nutritional quality of the fruits, thereby causing essential nutrient deficiencies to the consumers which can generally have a negative impact on their health (Rahman *et al.*, 2008). The effect of these artificial ripening agents on the food nutritional value and human health has drawn national and global attention. However, limited scientific information is available on the nutritional value of artificially ripened fruits, and their consequent effect on human health. The primary aim of this research was to evaluate the effect of Calcium carbide as a ripening agent, on the nutritional composition (proximate, mineral, vitamin and sucrose) and heavy metal content of orange and banana.

Materials and Methods

Sample Collection and Preparation

The unripe fruits (banana and oranges) were procured from Ojofu, Dekina Local Government Area, Kogi State, Nigeria. Unripe bananas and oranges with uniform peel colour and size were selected. The fruits were carefully separated from the bunch. Calcium carbide was procured from the local chemical vendor in the local market in Anyigba, Dekina Local Government, Kogi State, Nigeria. One (1) bunch of unripe banana containing 10 fingers of bananas and 10 unripe oranges were exposed to similar environmental conditions. The unripe fruits were separated into two equal batches, A and B. Batch A was allowed to ripen naturally, batch B was exposed to the same amount and type of ripening agent (Calcium carbide). Calcium carbide lumps, 50g each were placed in plastic bags containing the fresh unripe banana and orange samples separately. The ripening of treated bananas and oranges was carried out in a closed laboratory cupboard. Change in the skin-color from green to yellow was considered as the stage for the ripening of fruit (Gandhi *et al*., 2016). This was done to assess the ripening ability of different batches. Each treatment was carried out in duplicate. The physiochemical properties of the unripe and ripe bananas and oranges were determined according to standard methods of A.O.A.C. (2019).

Proximate Analysis

Determination of Moisture Content

Two (2) grams (g) of each processed samples were placed in a crucible and heated at 105^{0}C until a constant weight was attained. The moisture content of the sample was calculated as loss in weight of the original sample and expressed as percentage moisture content (Adeyemi *et al*., 2018).

$$\%\text{Moisture content} = \frac{W2 - W3}{W2 - W1} X\ 100 \ldots..1$$

Where: W1 = initial weight of empty crucible; W2 = weight of crucible + sample before drying; W3 = final weight of crucible + sample after drying

Determination of Ash Content

Approximately 2g of each sample were placed in a crucible ignited in a muffle furnace at 550 ^{0}C for 6 hours after which it was cooled in a desiccator (A.O.A.C., 2019; Bala and Bashar, 2017). It was weighed at room temperature to get the weight of the ash, using the formula:

$$\%\text{Ash content} = \frac{\text{Weight of Ash}}{\text{Weight of original sample}} X\ 100 \ldots..2$$

Determination of Crude Fibre

Crude fibre content was determined using the method of A.O.A.C. (2019). Five (5) grams of each of the sample and 200ml of 1.25% H_2SO_4 were heated for thirty (30) minutes and filtered with a Buchner funnel. The residue was washed with distilled water until it was acid-free. Two hundred (200) mills of 1.25% NaOH were used to boil the residue for thirty (30) minutes. It was filtered and washed several times with distilled water until it was perceived 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^{0}C in an oven overnight. After cooling in a desiccator, it was ignited in a muffle furnace at 550^{0}C for 90 minutes to obtain the weight of the ash. The percentage of crude fibre was obtained using this formula:

$$\%\text{Crude fibre} = \frac{W2 - W3}{W1} X\ 100 \ldots..3$$

Determination of Crude Fat

The estimation was performed using the Soxhlet extraction method. Ten (10) grams of each of the samples were weighed and wrapped with a filter paper and placed in a thimble. The thimble was covered with cotton wool and placed in the extraction column that was connected to a condenser. 200ml of n-Hexane was used to extract the lipid (A.O.A.C., 2019; Bala and Bashar, 2017). The fat content was calculated using the formula below:

$$\%\text{Fat content} = \frac{W2 - W3}{\text{Weight of sample}} X\ 100 \ldots..4$$

Where: W2 = Weight of filter paper and sample before extraction; W3 = Weight of filter paper and sample after extraction

Determination of Protein

Two (2) grams of each sample was weighed along with 20cm^3 of distilled water into a micro-Kjeldahl digestion flask. It was shaken and allowed to stand for some time. One tablet of selenium catalyst was added followed by the addition of 20cm^3 concentrated hydrogen tetraoxosulphate. The flask was heated on the digestion

block at 100^0C for 4 hours, until the digest became clear. The flask was removed from the block and allowed to cool. The content was transferred into $50cm^3$ volumetric flask and diluted to the mark with water. An aliquot of the digest ($10cm^3$) was transferred into another micro-Kjeldahl flask and placed in the distilling outlet of the micro-Kjeldahl distillation unit. A conical flask containing $5cm^3$ of boric acid indicator was placed under the condenser outlet. Sodium hydroxide solution ($10cm^3$, 40%) was added to the content in the Kjeldahl flask by opening the funnel stopcock. The distillation starts and the heat supplied were regulated to avoid sucking back. When all the available distillate was collected in $5cm^3$ of Boric acid, the distillation was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H_2S0_4; the end point was obtained when the colour of the distillate changed from green to pink. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C, 2019).

$$\%\text{Nitrogen} = \frac{Vs - Vb \times Nacid \times 0.01401}{W} X\ 100 \ldots..5$$

Where: Vs = Titre value of the sample; Vb = Volume of acid required to titrate; Nacid = Normality of acid; W = Weight of sample in grams

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 (Joseph and Abdullahi, 2016). The percentage carbohydrate was obtained using the formula:

% Carbohydrate = 100 - (%Protein + %Moisture + %Ash + %Fibre)….. 6

Mineral Analysis

The method of A.O.A.C. (2019) was employed for the determination of mineral content. One (1) gram of the pulverized sample was placed in a crucible and ignited in a muffle furnace at 550^0C for 6 hours. The resulting ash was dissolved in10ml 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 Ca while flame photometer was used for the determination of Na in the filtrate.

Vitamin Analysis

Determination of Vitamin A

The beta carotene was determined by soaking 1g of the sample (that is the paste or pulp of the fresh fruits) in 5ml of methanol for 2 hours at room temperature under dark condition in order to get a complete extraction. The beta carotene layer was separated using hexane through separating funnel. The volume was made up to 10ml with hexane and then this layer was again passed through Sodiumsulphonate through a funnel in order to remove any moisture from the layer. The absorbance of the layer was measured at 436nm using hexane as a blank (Aremu and Nweze, 2017). The beta carotene was calculated using the formula:

Beta-carotene (µg/100g) = Absorbance (436nm) × V × D × 100 × 100/W × Y ….. 7

Where: V = Total volume of extract; D = Dilution factor; W = Sample weight; Y = Percentage dry matter content of the sample

Determination of Vitamin C (Ascorbic Acid)

The method described by Okwu and Josiah, 2006 was used. Exactly 10g of the sample was extracted with 50ml EDTA/TCA (50g in 50ml of water). Extracting solution for 1 hour and filtered through a Whatman filter paper into a 50ml volumetric flask and made up to the mark with the extracting solution. Twenty (20) ml of the extract was pipette into a 250ml conical flask and 10ml of 30% KI was added and also 50ml of distilled water added. This was followed by 2ml of 1% starch indicator. This was titrated against 20% $CuSO_4$ solution to a dark end point. Vitamin C was determined using the formula:

$$\%\text{Fat content} = \text{Vit.C}\left(\frac{mg}{100}\right) = 0.88\ X\ \frac{100}{5}\ X\ \frac{vf}{20} X\ \frac{T}{1} \ldots..8$$

Where: Vf = Volume of extract, T = Sample titre- blank titre

Sucrose Analysis

The sucrose content was calculated by subtracting the reducing sugars originally present from the total reducing sugars. Exactly 110g of each sample was weighed and transferred into a 500ml volumetric flask. One hundred (100) ml of water was added and neutralized with 40% NaOH solution to phenolphthalein end point. Ten (10) ml of neutral lead acetate was added to the solution, shaken and left to stand for 10 minutes. 10% Potassium oxalate solution was added in small amounts until no further precipitation was observed. The solution was made up to volume, mixed well and filtered. The filtrate was then transferred into a 50ml burette having an off-set tip.

Preliminary Titration

Five (5) ml of each Fehling A and B solutions was pipette into a 250ml conical flask. Ten (10) ml of water and glass beads (anti-bombing) was added to the solution and mixed. The solution was heated to boiling. Three (3) drops of methylene blue indicator were added and the filtrate (sugar solution) was run into the solution. Sugar solution was added continuously and stirred until the blue colour changed to a brick-red end point.

Final Titration

Five (5) ml of each Fehling A and B solutions was pipette into a 250ml conical flask. About 0.05-1.0ml of the sample solution less than titre value of the preliminary titration was added. The flask was heated to boiling. Three (3) drops of methyl blue indicator was added. The titration was complete within one (1) minute

by the addition of 3 drops of sugar solution at a time, until the indicator was decolourized. At the end point, the boiling liquid assumed the brick red colour.

Statistical Analysis
Data collected were subjected to analysis of variance (ANOVA) using the SPSS version 21. Significant means ($p \leq 0.05$) were separated using Duncan New Multiple Range Test (DMRT).

Result s and Discussion
Results
Proximate Analysis
Results from proximate analysis of the orange (Table 1) revealed that there was a significant difference ($p<0.05$) in the moisture, fat and protein levels in the control (naturally ripened orange) and sample (Calcium carbide induced, ripened orange). Also, there was no significant difference ($p<0.05$) in the ash, fibre and carbohydrate levels in the control and sample. The control had the highest concentration in fat, carbohydrate and protein, while the sample had the highest concentration in moisture, ash and fibre. Similarly, there was a significant difference ($p<0.05$) in the moisture, fat and protein level of the Banana. Likewise, there was no significant difference ($p >0.05$) in the ash, fibre and carbohydrate level. The control had the highest concentration in fat and protein while the sample had the highest concentration in moisture, ash, fibre and carbohydrate.

Mineral Analysis
As shown in Table 2, significant difference ($p<0.05$) was observed in the mineral level (Sodium and Calcium) of the control and sample. The control had the highest concentration.

Vitamin and Sucrose Analysis
Results shown in Table 3 below reveals that there was a significant difference ($p<0.05$) in the vitamin level (C and A) in the control and sample. The control had the highest concentration across.

Heavy Metal Analysis
Results shown in Table 4 below reveals that there was no significant difference ($p>0.05$) in the heavy metal level (Lead and Arsenic) between the control and sample. The sample however, had traces of heavy metal which was not present in the control.

Discussion
After ripening with Calcium carbide, the moisture, ash and fibre levels increased in both banana and orange. This finding is consistent with the findings of Sogo-temi *et al*. (2014), who investigated the nutritional and metal composition of bananas using biological and chemical ripening methods (*Musa spp*.). Furthermore, the naturally ripened fruits had more protein than the Calcium carbide-ripened ones. This is also in line with the findings of Sogo-temi *et al*. (2014), who found a decrease in protein concentration following ripening with Calcium carbide. This decrease could be the result of a considerable drop in nitrogen during rapid ripening.

After ripening with Calcium carbide, mineral analysis revealed a decrease in Calcium levels. However, this result is contradicted by the findings of Bawa *et al*. (2020) and Oguntade and Fatumbi (2019). Calcium levels increased after ripening with Calcium carbide according to these studies. The current study's contradicting findings are intriguing and warrant additional exploration. Nonetheless, the findings of Bawa *et al.* (2020) on Sodium reduction in Calcium carbide-ripened fruits are similar to those of the current study. In comparison to naturally-ripened fruits, Yeasmin *et al.* (2019) found that carbide-ripened fruits had lower Sodium levels also. However, there was a discrepancy with Iroka *et al.* (2016), who showed an increase in Sodium content for Calcium carbide-ripened fruits.

Furthermore, there was a decrease in vitamin A and C content in the fruits ripened with Calcium carbide. Similarly, there was a decrease in sucrose level. The naturally ripened fruits were seen to contain more vitamin A and C than fruits ripened with Calcium carbide. This result supports the work done by Majagi and Jabannavar (2019) on the comparison of vitamin C levels in naturally-ripened and artificially ripened mangoes. The result showed that the naturally-ripened mangoes had more vitamin C content than artificially ripened mangoes. The findings back up that of Gbakon *et al*. (2018) on the effects of Calcium carbide treatment on some physicochemical parameters of broken and mummy mango fruits. The results showed that carbide-ripened mangoes had a larger drop in vitamin C content than naturally-ripened mangoes. The larger drop was attributed to the faster action of Calcium carbide throughout the ripening phase according to the researchers. The presence of arsenic and lead in the Calcium carbide-ripened fruit is consistent with other studies (Bawa *et al*., 2020; Abbas *et al*., 2021) that have obtained similar results. Industrial Calcium carbide has been shown to have high levels of arsenic, lead and phosphorus compounds, all of which are harmful to humans and can contaminate artificially ripened crops according to reports (Muanya, 2019). This explains why the artificially ripened fruit in this study contained arsenic and lead.

Conclusion
The use of Calcium carbide as an artificial ripening agent causes the vital nutritional content of fruits to decrease. Calcium carbide treatment reduced the amount of protein, fat, vitamin A and C, Calcium, Sodium, and sucrose in the fruits studied. On the other hand, it increased the moisture, ash, and fibre content of the fruits. Although the carbohydrate content of bananas ripened with Calcium carbide increased, the carbohydrate content of carbide ripened oranges decreased. Calcium carbide also resulted in the presence of traces of Ld and As in the samples. On a general note, this study found that using Calcium carbide as a ripening agent results in a significant loss of fruit nutrients.

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Table 1: Proximate composition of artificially (sample) and naturally (control) -ripened fruits

	Orange		Banana	
(%)	**Control**	**Sample**	**Control**	**Sample**
Moisture	84.71±0.01[a]	85.25±0.07[b]	44.46±0.08[a]	46.10±0.00[b]
Ash	0.18±0.04[a]	0.25±0.00[a]	0.45±0.07[a]	0.63±0.04[a]
Fibre	0.49±0.01[a]	0.52±0.00[a]	0.07±0.01[a]	0.09±0.00[a]
Fat	0.15±0.01[b]	0.08±0.00[a]	1.29±0.01[b]	1.02±0.03[a]
Carbohydrate	13.61±0.01[a]	13.58±0.03[a]	48.75±0.01[a]	50.43±0.64[a]
Protein	0.88±0.00[b]	0.29±0.04[a]	4.98±0.06[b]	2.24±0.00[a]

Values with different superscript alphabet across the rows under each fruit (separately) are considered significantly different (p<0.05). Values are Mean ± SD, Using Independent Sample t-test

Table 2: Mineral composition of artificially and naturally ripened fruits

	Orange		Banana	
(ppm)	**Control**	**Sample**	**Control**	**Sample**
Sodium (Na)	50.30 ± 0.28[b]	39.55 ± 0.21[a]	70.70 ± 0.28[b]	59.10 ± 0.00[a]
Calcium (Ca)	111.10 ± 0.14[b]	97.35 ± 0.07[a]	168.25 ± 0.07[b]	155.05 ± 0.07[a]

Values with different superscript alphabet across the rows under each fruit (separately) are considered significantly different (p<0.05). Values are Mean ± SD, Using Independent Sample t-test

Table 3: Vitamin and sucrosecomposition of artificially (sample) and naturally (control) ripened fruits

	Orange		Banana	
	Control	**Sample**	**Control**	**Sample**
Vitamin A (µg/100g)	565.33±8.22[b]	363.10±0.00[a]	282.74±4.21[b]	142.86±0.00[a]
Vitamin C (mg/100g)	102.07± 0.23[b]	73.92± 0.46[a]	43.47± 0.23[b]	25.07± 0.00[a]
Sucrose (%)	42.99± 0.18[b]	36.95± 0.23[a]	76.28± 1.33[b]	55.15± 0.37[a]

Values with different superscript alphabet across the rows under each fruit (separately) are considered significantly different (p<0.05). Values are Mean ± SD, Using Independent Sample t-test

Table 4: Heavy metalcomposition of artificially (sample) and naturally (control) ripened fruits

	Orange		Banana	
(ppm)	**Control**	**Sample**	**Control**	**Sample**
Lead (Pb)	0.00 ± 0.00[a]	0.015±0.017[a]	0.00 ± 0.00[a]	0.023±0.026[a]
Arsenic (As)	0.00 ± 0.00[a]	0.001±0.001[a]	0.00 ± 0.00[a]	0.001±0.002[a]

Values with different superscript alphabet across the rows under each fruit (separately) are considered significantly different (p<0.05). Values are Mean ± SD