The Effect of Selected Ripening Agents on the Physico-Chemical Properties and Sulphide/Sulphate Distribution of Banana (Musa Sapientum) Fruit

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Authors' contributions

This work was carried out in collaboration between both authors. Author CAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author CIU managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARJA/2021/v14i130114

Received 01 November 2020
Accepted 06 January 2021
Published 12 March 2021

ABSTRACT

This study aimed at evaluating the effect of two ripening agents (calcium carbide and kerosene fumes) on the physiochemical properties and sulphide/sulphur distribution of banana fruit. Freshly unripe banana fruit were treated with calcium carbide powder and kerosene fumes and ripened within 48 hrs. Fruit samples were also ripened naturally and served as control. The samples were analyzed for physicochemical properties and sulphur/sulphate distribution (outer and inner). The result of physicochemical analysis revealed a significant (p<0.05) decrease in pH (5.43-4.75), total titratable acidity (TTA) (5.03-0.47%), moisture (75.87-67.13%), carbohydrate (11.14-5.09%) and vitamin C (0.27-0.002mg/100g) with an increase in total soluble solids (2.00-19.30°Brix) following ripening process. Amongst the ripened fruits, fruits ripened with calcium carbide had highest TTA (1.63%) and moisture (74.75%). Accelerated ripened banana fruits had low pH>5 and higher TSS
than naturally ripened sample. The concentrations of sulphur/sulphide (0.29-1.85mg/kg) were below the limit of 50 mg/kg indicating that the fruits were still safe for consumption against health threats posed by high concentrations of sulphate/sulphide. This study therefore quantified the changes in physicochemical properties of artificially ripened banana fruits and their possible health hazards. The study is very useful particularly in relation to the health hazards associated with chemical treatment for banana ripening. This will be useful to banana fruit sellers as it may help to optimise the ripening practices which may lead to reduce the safety and health concerns of the consumer.

Keywords: Accelerated ripening; calcium carbide; kerosene fumes; banana.

1. INTRODUCTION

Fruit ripening is a natural process in which fruits go through various physiological, biochemical and molecular processes which makes the fruit become sweet, colored, soft and suitable for eating [1]. It involves coordination of different metabolism with activation and deactivation of various genes leading to change in color, increase in sugar content, decrease in acidity, softening of fruit and increase in flavor and aroma [2]. The ripening process of fruits can be naturally or accelerated using different chemical agents such as ethanol, methanol, methyl jasmonate, ethylene glycol, ethephon and calcium carbide. Accelerated ripening agents are used to speed up the ripening process which is required for commercial purposes. The use of kerosene fumes or lantern in initiating the ripening process of fruits has also been on the increase recently. Several studies on the effect of these ripening agents has been carried and reports have been made that possible health hazards are caused by direct exposure or direct consumption of artificial ripening agents [3,4].

Banana (Musa sapientum) is a monocotyledonous, perennial herb within the order Zingiberales, and the family Musaceae [5]. The tree grows up to a height of about 2-8m with leaves of about 3.5m in length. The pseudostem of banana produces a single bunch of banana before dying and replaced by new pseudostem. The fruit grows in hanging cluster, with twenty fruits to a tier and 3 – 20 tiers to a bunch of varying size, color, and firmness, with soft flesh which is green when unripe, yellow when ripe. Banana is one of the most popular fruits in the world known to contain essential nutrients and have protective impact on human health. Banana fruit has been reported by Mohapatra et al. [6] to not only be a rich source of carbohydrate, antioxidants, but also a good source of mineral, especially potassium and iron, an ideal food for weaning mother and infants. Bananas are identified as relatively rich in pyridoxine which could further protect the body against cancer of the oesophagus [7]. Bananas can be eaten raw, blended into a juice or eaten as a fruit salad or smoothie with other fruits. They are also processed into flour and blended with some legume based products which could be served as excellent baby food and snack food.

Artificial ripening agents are chemicals that can be used to ripen fruits. Various artificial methods of fruit ripening have been observed mostly to meet consumers’ demand and other economic factors. People consume fruits ripened with hazardous chemicals like calcium carbide which poses great health risks to consumers [3]. Banana is one of the most widely consumed fruit crops in the world. As a result of rising population and need to meet the growing demand of the population, different ripening agents are used to accelerate the ripening process of the banana fruits. In Nigeria, ripening agents such as calcium carbide, African bush mango fruit and leaves, yellow pawpaw leaves, potash, ash and lantern or kerosene fumes have been commonly used for banana fruit ripening. The use of these ripening agents speeds up the rate of process, of the fruit which in turns affects the nutritional quality and safety of the fruit [1]. Several studies have reported that the consumption of accelerated ripened fruits is extremely hazardous to health, because it affects the nervous system, irritate the mucosal tissues of the abdominal region [8], causes stomach disorder [9] and kidney failure [10, 11]. The aim of this study is to evaluate the effect of accelerated ripening agents (calcium carbide and kerosene fumes) on the physicochemical properties, sulphur and sulphate distributions of banana fruit in order to identify any change in nutritional values of artificially ripened banana fruit and also any possible health hazard associated with its consumption.
2. MATERIALS AND METHODS

2.1 Identification and Collection of Samples

Banana (Musa sapientum) fruits were harvested from the Rivers State University school farm in the month of April 2019. Identification of the materials was done by a plant scientist in the Department of Crop and Plant Science, Rivers State University to ascertain that the right materials were used for the research. All chemicals used were of analytical grade.

2.2 Banana Ripening Processes

2.2.1 Ripening with calcium carbide

Fresh unripe banana fruits were placed in a container (25 L rectangular) of room temperature. Then 10 g of calcium carbide powder was placed opposite the fruits in the container. The container was opened after 48 hrs and all the samples were fully ripened as shown in Fig. 1.

2.2.2 Ripening with kerosene fumes

Fresh unripe banana fruits (Fig. 2) were placed in an airtight container (25 L round container) of room temperature. A flat-wick lighted kerosene lantern was placed at the center of the container and was allowed to burn until the oxygen inside was exhausted (the light went off in 10 min). The samples were kept in the container for 24 hrs and then exposed to open air for another 24 hrs. All the samples were fully ripened after the 24 hrs exposure to air as shown in Fig. 3.

2.2.3 Naturally ripened banana

Fresh unripe banana fruits obtained from the parent stock (tree) were allowed to naturally ripen at room temperature condition. Ripening took place after three weeks as shown in Fig. 4.

2.3 Physicochemical Analysis

Moisture content, pH and Vitamin C content of the ripened banana fruits was carried out using the AOAC [12] method. Total available carbohydrate was determined using the manual Clegg Anthrone method of Osborne and Vogt [13]. The hand held sugar refractometer was also used in determining the total soluble solids (°Brix) of the fruits. Total titratable acidity was determined by weighing 6 g of the sample into a beaker and titrating each sample with 0.1 N NaOH to an end point and then calculated [14].

2.4 Sample Preparation for Sulphide and Sulphate Analysis

The banana fruit samples were washed with distilled water and raised with deionized water. The outer layer was sliced in bits and grind to form a wide area concentration placed in conical flask and well labelled. The inner layer part of the seed (endosperm) was cut in cubes and grind. A known weight (2.0 g) was measured into a conical flask and 20.0 ml of deionized water set in mechanical shaker for 30 minutes. The filtrate was set taken to Hach Spectrophotometer for analysis as described below.

2.4.1 Sulphide analysis (Methylene blue method ASTM D4658)

Sulphide content was determined using the methylene blue method as described by Moest [15].

2.4.1.1 Reagents

a. Sulphide reagent 1 and 2.

b. Sodium sulphide saturated solution: Hundred grams (100 g) of sodium sulphide hydrate was dissolved in 100 ml of distilled water.

c. Sulphide anti-oxidant buffer (SAOB): Eighty grams (80 g) of sodium hydroxide, 35 g of ascorbic acid and 67 g of EDTA was dissolved in 600 ml of distilled water and made up volume with distilled water.

d. Sodium sulphide stock solution: One millilitres (1.0 ml) of the saturated solution described above was pipette into 50 ml of SAOB and diluted to 1 litre distilled water.

2.4.1.2 Procedure

The stored program number for sulphide was entered. The display showed in mg/l S\textsubscript{2} and the zero icon. The sample was filtered since it was turbid. A clean cuvette was filled with 25 ml of the sample to be analyzed. Another sample cuvette was filled with 25 ml of distilled water which served as the blank. One millilitres (1 ml) of reagent 1 was added to both the blank and sample and swirl. The timer was pressed and a five-minute reaction programe began. The cuvettes were then allowed to stand undisturbed. After the timer beeped, the blank cuvette was inserted into the sample holder and covered tightly. The ZERO
icon was pressed. The sample was inserted and the READ icon pressed. The result displayed in mg/l S\textsubscript{2}.

2.4.1.3 Calibration procedure

Four different concentrations were prepared.

Standard 1: Five millilitres (5.0 ml) of sodium sulphide stock solution and 50 ml of sulphide antioxidant buffer were measured and diluted to 100 ml with distilled water (0.1 ppm).

Standard 2: One millilitre (1.0 ml) of sodium sulphide stock solution and 50 ml of sulphide antioxidant buffer were measured and diluted to 100 ml with distilled water (0.02 ppm).

Standard 3: Two millilitres (2.0 ml) of calibration standard 1 was measured and 50 ml of sulphide antioxidant buffer added, diluted to 100 ml with distilled water (0.002 ppm).

Standard 4: One millilitre (1.0 ml) of calibration standard 1 was measured and 50 ml of sulphide antioxidant buffer added, diluted to 100 ml with distilled water (0.001 ppm).

A calibration curve was prepared using concentration and absorbance reading.

2.4.2 Sulphate analysis (Turbidometric test method ASTM D516-07)

Sulphate content of the fruits was determined using the methylene blue method as described by Tabtabai [16]. A user-enter calibration is necessary to obtain the most accurate result. The stored program number for sulfate SO\textsubscript{4} was entered. The display showed in Mg/L SO\textsubscript{4} and the ZERO icon. A clean sample cell was filled with 10 ml sample to be analyzed. The content of one sulfaver 4 sulfate reagent powder pillow was added to the sample cell. The cell was capped and inverted several times to mix. Press timer A 5 minute reaction program will begin. The cell was allowed to stand undisturbed. After the timer beeped, a second sample cell was filled with 10 ml of sample (the blank). The blank was placed into the cell holder. The sample cell was tightly covered with the instrument cap. The ZERO key was pressed and then display showed 0mg/L SO\textsubscript{4}. The prepared sample was placed into the cell holder. Tightly cover the sample cell with the instrument cap within five minutes after the timer beeps. The READ key was pressed and result was displayed in mg/l SO\textsubscript{4}.

2.4.2.1 Calibration procedure

One thousand parts per million (1000 ppm) sulphate standard solution was prepared by dissolving 0.0256 g of oven dried magnesium sulphate salt and diluted to 100 ml with deionized water. A 10 ml pipette was used to add 1, 2, 3, 4, 5 and 6 ml of the standard to the 100 ml volumetric flask, made up to mark with deionized water (This Represents 10, 20, 30, 40, 50, and 60 ppm of the sulphate standards respectively). This was stoppered and mixed well. The instrument was zeroed with water while each standard was analyzed using the photometer. The sulphate concentration increased by 10 mg/l for each 10 ml of standard added. The Calibration curve was plotted of absorbance against SO\textsubscript{4} concentration.

2.5 Statistical Analysis

All experiments and analysis were carried out in triplicates. The mean and standard deviation values were calculated. Data were subjected to Analysis of Variance (ANOVA). Means were separated using Tukey’s multiple comparison test, and significance accepted at p≤0.05 level. The statistical package in Minitab 16 computer program was used.
3. RESULTS AND DISCUSSION

3.1 Physico-chemical Properties of Banana Fruit as Affected by Ripening Agents

The physico-chemical properties of banana fruit as affected by ripening agents is shown in Table 1. Moisture content of the banana fruit ranged from 67.13-74.75% with unripened sample recording the highest and naturally ripened sample as lowest. There was a significant (p<0.05) difference in the moisture content of all the banana samples except for calcium carbide and kerosene fumed ripened samples. The moisture decreased during ripening and the decrease was more pronounced with the naturally ripened banana sample. This could be due to the fact that these ripening agents (calcium carbide and kerosene fume) probably have interfered with biochemical pathways of banana during ripening thereby resulting to higher moisture content than the natural ripened banana fruit [3]. Similar finding was also reported by Orisa and Usoroh [16]. High moisture content of the ripened banana using accelerated ripening agents will expose it to increased microbial spoilage and short shelf life than the naturally ripened banana thereby leading to deterioration.

![Kerosene fumed banana](image1)

Carbohydrate content of the banana fruits ranged from 5.09-11.14% with unripe sample recording the highest while calcium carbide ripened sample, the lowest. Carbohydrate contents of the unripe and naturally ripened samples were significantly (p<0.05) similar. Ripening of banana with calcium carbide and kerosene fumes were observed to affect the carbohydrate content of banana differently. Higher carbohydrate content of unripened banana sample as compared to ripened samples is due to the hydrolysis of carbohydrates into sugars during ripening [1]. The finding of the present study strongly agrees with the study of Orisa et al. [3] for mango and avocado ripened with calcium carbide and kerosene fumes.

![Naturally ripened banana](image2)

Total soluble sugars (TSS) of the banana fruits ranged from 2.00°brix in unripe banana to 19.65°brix in kerosene fumed banana. TSS of banana ripened with calcium carbide and kerosene fume were significantly (p<0.05) similar. Ripening was also observed to significantly (p<0.05) increased the TSS content of the fruits and this was more pronounced with the banana ripened with accelerated agents. Accelerated ripening with calcium carbide and kerosene fumes may probably have interfered with some biochemical pathways during ripening thereby increasing the TSS as compared to naturally ripened fruit [3]. Higher TSS of ripened fruits is associated with the breakdown of pectin and conversion of carbohydrate and conversion into simple sugars during storage caused by metabolic activities of the tissues. Similar findings was also observed by Kafkas et al. [17] who reported that fructose, the main sugar, increased during ripening of strawberry.

![Physico-chemical properties of banana as affected by ripening agents](table1)

| Samples | Moisture (%) | Total soluble solids (°Brix) | Total Available Carbohydrate (%) | Vitamin C (mg/100g) | Total titratable acidity (%) | Ph |
|---------|--------------|-----------------------------|---------------------------------|---------------------|----------------------------|----|
| CCB     | 74.74±1.41a  | 19.30±0.14a                 | 5.09±0.79a                      | 0.003±0.00a         | 1.63±0.04c                 | 4.75±0.07c |
| KFB     | 73.16±1.41a  | 19.65±0.78a                 | 5.44±1.05a                      | 0.003±0.00a         | 0.57±0.01c                 | 4.75±0.04c |
| NRB     | 67.13±3.75a  | 12.05±0.07a                 | 9.90±1.41a                      | 0.002±0.00a         | 0.47±0.02b                 | 5.01±0.002 |
| UB      | 75.87±0.49a  | 2.00±0.00c                  | 11.14±1.61a                     | 0.27±0.37a          | 5.03±0.04a                 | 5.43±0.04c |

Values are means ± standard deviation of triplicate samples. Mean values bearing different superscript in the same column differ significantly (P<0.05)

Key: CCB=Calcium carbide Banana, KFB=kerosene fume Banana, NRB=naturally ripened Banana, UB=unripe Banana
Vitamin C content of the banana fruits ranged from 0.002mg/100g in naturally ripened banana fruit to 0.27mg/100g in unripened sample. The study showed a decreasing trend of vitamin C on ripening. However, vitamin C content of the fruits were not significantly (p<0.05) affected by accelerated ripening agents. Vitamin C content of the banana fruit during ripening were low when compared to values of 175-1071.66ppm obtained for banana ripened with ethephon, kerosene fume, calcium carbide and ethylene glycol [2]. These differences could be due to the analytical procedure and difference in habitats of the fruits.

PH of the ripened banana fruits ranged from 4.75-5.43. The unripened samples had higher pH values than the ripened sample. pH of banana samples ripened artificially was significantly (p<0.05) similar and showed the lowest. This shows a reduction in pH on accelerated and natural ripening. These reductions are due to biochemical reactions which might have taken place in the calcium carbide and kerosene ripened fruits. Natural ripening of fruit involves multiplicity of biochemical pathways. Ripening of banana fruit with kerosene fume and calcium carbide may probably have interfered with these biochemical pathways thereby reducing its pH [3]. Zenebe et al. [18] also reported a reduction in pH of banana fruits ripened through kerosene smoking systems. The study also showed that pH of banana fruit during ripening are slightly acidic in nature with pH of 5. It has been reported that fruits with a pH below 5 can trigger dental erosion [19]. This may suggest that banana fruit ripened with calcium carbide and kerosene fumes will accelerate dental erosion when consumed as their pH were below 5.

Total acidity of banana fruit from the study ranged from 0.47-5.03% with unripe sample having the highest and naturally ripened sample as lowest. TTA of kerosene fumed banana and naturally ripened samples were significantly (p<0.05) similar. The result showed that ripening reduced the total titratable acidity of the fruits and this was more pronounced with natural ripened and kerosene fumed fruits. The decrease in acidity is due to susceptibility of the predominant acid to oxidative destruction as influenced by the ripening environment. It might also be due to their utilization as substrates for respiration [20]. Similar observation was reported by Haejin et al. [21] who stated that organic acids increased mostly during the early stages of fruit growth and decreased until fruits were fully ripened. Fruits with high acidity have been reported to have implications to dental health as they might cause dental erosion, especially among children [22]. Acidity levels of the calcium carbide banana fruit were high which indicates that is not advisable for consumption safe.

3.2 Sulphate and Sulphide Distribution (mg/kg) of Banana as Affected by Ripening Agents

The sulphate and sulphide distribution (mg/kg) of banana as affected by ripening agents is shown in Table 2. Sulphate (outer and inner) distribution ranged from 1.13-1.85mg/100g and 0.59-1.58mg/100g, respectively. On the other hand, sulphide (outer and inner) distribution ranged from 0.17-1.39mg/100g and 0.00-0.29mg/100g, respectively. Outer sulphide and sulphur contents of the calcium carbide banana fruit was significantly (p<0.05) higher than the kerosene fumed and unripe samples. Outer sulphur distribution of calcium carbide and kerosene fume banana samples were significantly (p<0.05) different. Sulphur and sulphide concentrations were higher for accelerated ripened banana samples and this was pronounced with calcium carbide samples. Similar observations were reported by Orisa and Usoroh [16] for pawpaw fruit ripened with calcium carbide and kerosene fume. The higher concentrations of sulphur and sulphide as observed with the calcium carbide ripened banana fruit could be attributed to the diffusion of sulphite and sulphate from calcium carbide to fruit pulp and skin during the ripening processes.

Table 2. Sulphur and sulphide distribution (mg/kg) of banana as affected by ripening agents

| Samples | Sulphur (Outer) | Sulphur (Inner) | Sulphide (Outer) | Sulphide (Inner) |
|---------|----------------|----------------|----------------|----------------|
| CCB     | 1.85±0.00      | 1.58±0.18      | 1.39±0.19      | 0.29±0.01      |
| KFB     | 1.19±0.12      | 1.55±0.00      | 0.48±0.04      | 0.18±0.10      |
| UB      | 1.13±0.04      | 0.59±0.11      | 0.17±0.00      | 0.00±0.00      |

Values are means ± standard deviation of triplicate samples. Mean values bearing different superscript in the same column differ significantly (P<0.05)

Key: CCB=Calcium carbide Banana, KFB=Kerosene fume Banana, UB=Unripe Banana
Enam et al. [23] reported kerosene to contain impurities such as sulfur, aromatic compounds and hydrocarbons. This may be the reason for increase in sulphur and sulphide concentration of the banana fruits during accelerated ripening process thereby posing serious health risk for consumers [3]. Several studies have been carried out on the health hazards of excessive sulphur intake. Islam et al. [2] reported that inhalation of 4-6 ppm of sulphur dioxide for 10 min decreases airway conductance of a healthy person. They also stated that acute exposure to high concentration of sulfur dioxide can also cause pulmonary injuries which sometimes lead to death. The FAO/WHO [24] reported that the maximum level of sulphites permitted in fresh fruits with surface treatment is 50mg/kg. In this study, the concentrations of sulphide and sulphate of the banana fruits ripened with accelerated agents was below this limit indicating that the fruits are still safe for consumption against health threats posed by high concentrations of sulphate.

4. CONCLUSION

This study showed that moisture contents, total soluble solids (°Brix) and total titratable acidity of banana samples ripened with accelerated ripening agents were higher than the naturally ripened sample. Physicochemical parameters of the fruit also showed that the pH of accelerated ripened banana samples was below 5. Carbohydrate content of accelerated ripened samples was lower than the naturally ripened samples. Ripening agents used in this study had no significant effect on the vitamin C, moisture, TSS and pH content of the banana fruits. Among the two ripening agents studied, calcium carbide ripened banana contained significantly high amount of sulphate and sulphide than kerosene fumed sample. However, the sulphite/sulphate concentrations were below the maximum permissible limit indicating that the banana fruits were still safe for consumption against health threats posed by high concentrations of sulphate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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