



Research Article

Assessment of Heavy Metal Residue, Physicochemical Properties, and Sensory Properties of CaC₂ Treated Banana

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ARTICLE INFO	ABSTRACT
<p>Article history Received: 02 Apr 2022 Accepted: 05 Jul 2022 Published: 30 Sep 2022</p> <p>Keywords Calcium carbide, heavy metals, food safety risk, sensory evaluation</p> <p>Correspondence Mohammad Gulzarul Aziz ✉: aziz_ftri@bau.edu.bd</p> <p> OPEN ACCESS</p>	<p>This study assessed the food safety risks associated with bananas ripened by CaC₂. For ripening, calcium carbide concentrations were used per kilogram of bananas (1% and 2% of calcium carbide solution; 5 g and 10 g of calcium carbide powder) and compared results of artificially ripened fruits with control samples. Heavy metals like arsenic and calcium and phosphorous content in market bananas were found higher than in other treated samples. Total sugar, reducing sugar, TSS, pH, titrable acidity, vitamin C, and carotenoid were lower in the market sample than in other samples. Moisture content increased while total sugar, reducing sugar, TSS, pH, titrable acidity, vitamin C and carotenoid decreased with increasing CaC₂ concentration. Other than titrable acidity, pH, total sugar, and TSS were negatively correlated with the heavy metals. Sensory properties decreased with increasing CaC₂ concentration. In conclusion, the market sample contained more health risks compared to the calcium carbide ripened banana assessed in this study.</p>
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Introduction

Around the world banana (*Musa spp*) is one of the most consumed tropical fruits and has huge popularity as part of the everyday diet around the world. Countries in Asia, Latin America and Africa are the major cultivators of this fruit (Song et al., 2015). It is from the Musaceae family that is commonly used as one of the main staple food or a nutritious supplement for around 400 million people (Pongprasert et al., 2020; Vaidya et al., 2016). Many studies have found that Banana contains a promising amount of vitamins A and C, carbohydrates, and elements like phosphorous, potassium, magnesium, selenium and iron. Banana is also recommended for diet because of their low content of sodium chloride (Kumar et al., 2012; Abdul-Rahaman et al., 2015).

When bananas reach the mature green stage, they are harvested commercially and ethylene treatment is induced to reach the fully ripened stage prior to marketing. The optimum conditions for ripening of bananas would be 20°C temperature and 90% of relative humidity with a properly ventilated post-harvesting storage area. These conditions are necessary to achieve desired quality attributes for the market (Hailu et al., 2013). It has been observed that artificial ripening is being used more frequently by traders to meet the desired market standard, due to various limitations of natural ripening, lack of uniformity in peel color and delayed ripening (Bhattarai and Shrestha, 2005). These situations make artificial ripening a necessary practice in banana ripening. Artificial ripening has been shown in numerous studies to have a significant impact on the physicochemical properties,

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and nutritional and sensory quality of banana fruit (Maduwanthi & Marapana, 2021). Bananas treated with forced ripening agents were reported to have possessed low nutritional value because of the substances in ripening agents which include vitamin c, carotene and mineral reduction (Hakim et al., 2012). Significant changes in the sugars, total soluble solids, organic acids, aroma volatiles, and titrable acidity have also been reported. Among the various chemical agents that can aid ripening, calcium carbide, ethephon, and ethylene gas are mostly reported (Maduwanthi & Marapana, 2021).

Calcium carbide (CaC₂) is the most extensively used artificial ripening agent, owing to its low cost and availability. It chemically interacts with water to make acetylene (Gupta, 2017). Because it contains traces of heavy elements like arsenic and phosphorus, calcium carbide for food treatment is regarded as particularly toxic. Health hazards like dizziness, headache, memory loss, mood disturbances, sleepiness, and seizures are the result of the consumption of calcium carbide inoculated fruits (Adeyemi et al., 2018). In a recent study, researchers noticed that the consumption of artificially ripened fruits with calcium carbide negatively alters the female reproductive physiology with the signs of oestrogenic disruptions which is an alarming issue (Bafor et al., 2019). Though chemical ripening has adverse effects, Bangladeshi retailers and farmers use ripening agents to expedite the ripening of fruits; similar practices are also followed in most developing countries like Pakistan, Ghana, Cameroon and Nigeria (Adeyemi et al., 2018).

Considering the allover scenario, this study aims to compare food safety risks associated with banana ripened with traditional and chemical methods using calcium carbide. Specifically, the estimation and detection of hazardous residues and physicochemical properties, along with the assessment of the food safety risk of the chemicals identified in the ripened banana using a variety of methods. Finally, recommend a suitable strategy for fruit ripening for small-scale dealers and assess its impact on banana quality.

Materials and Methods

Materials

The bunches of unripe Sabri bananas were collected from the Madhupur town in Mymensingh. CaC₂ was collected from Dhaka. CaC₂ powder (5 and 10g) and solutions (1 and 2%) were prepared in the laboratory. One set of ripened bananas was collected from the local market in Mymensingh as a reference. High-density polythene bags and cartons were used for the packaging and storage of the sample. All the chemicals and reagents used were of analytical grade.

Sample preparation for ripening procedure

Six different samples were prepared for the ripening procedure: Control banana sample (T₁), bananas treated with 1% CaC₂ (T₂), bananas treated with 2% CaC₂ (T₃), bananas treated with 5gm of CaC₂ powder (T₄), bananas treated with 10gm of CaC₂ powder (T₅) and bananas obtained from the market as a reference sample (T₆). 1% and 2% CaC₂ solutions were prepared by dissolving 10gm and 20gm of CaC₂ powder in 1-liter water respectively. Each treatment was performed using a bunch of bananas that weighed 1kg. For treatments with CaC₂ solutions, sample bananas were dipped into the solutions for 30 minutes, followed by removing the excess moisture from the exterior by a moving fan. After that, the samples were placed separately in 1 kg cartons and stored in a room at ambient temperature (30-37°C) for further analysis. For the samples treated with CaC₂ powder, they were placed in a separate 1 kg container with 5 gm of CaC₂ powder sachet placed in a single layer and for T₅ samples, 10 gm of CaC₂ powder were placed in a double layer (5 g sachet per layer). The cartons were kept with the previous samples. The control samples were also placed with the treated samples for future analysis.

Wet-digestion of samples for the determination of residual arsenic, calcium and phosphorus

Wet digestion of the samples was done by a previously established method (Akinyele & Shokunbi, 2015) with slight modification and the wet digested samples were cooled and made to a known volume by using double distilled water.

Determination of arsenic and calcium residue by an Atomic Absorption Spectrophotometer

The residual arsenic and calcium were determined in triplicates by using an atomic absorption spectrophotometer (Ackah et al., 2014; AOAC, 2003) at Food Quality and Safety Laboratory, IIFS, BAU, Mymensingh. The residual arsenic and calcium in the sample were calculated using the following equation:
5g of banana fruit part contain arsenic/calcium= X ppb
100 g of banana fruit part contain arsenic/calcium= (X×100)/5 = 20 X ppb

Determination of the phosphorous residue by UV spectrometer

The phosphorous residue was determined in the wet digested samples by using a UV Spectrophotometer (Shyla, & Nagendrappa, 2011) and the readings were taken at 890nm.

Estimation of physicochemical characteristics

Moisture content

The moisture content was determined by using a hot air oven and muffle furnace (AOAC, 2002).

Total soluble solids (TSS)

The TSS of the samples was recorded by using a handheld refractometer (0-32°B). The reading thus obtained was corrected for temperature variation to 20°C as per the International Temperature Correction Table (Horwitz, 1980; RMNA & Guleria, 2009) and the results were expressed as °Brix (Ali et. al., 2018).

Titration acidity

Titration acidity was determined using the titration method with the following formula and expressed as percent citric acid on a fresh weight basis (AOAC 2002).

$$\% \text{ Titration acidity} = \frac{\text{titre} \times \text{normality}}{\text{weight of sample}}$$

pH

The pH of the banana was determined by using a pH meter at an ambient temperature.

Colour

The colour of the banana sample was observed at room temperature by using a Chroma Meter (CR-400 HEAD). Total colour change was obtained by measuring ΔE^* (Shiraj-Um-Monira et al., 2019). The following equation is used to determine the colour deviation-

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

L^* = brightness from black (0) to white (100).

a^* = red-green colour. (Positive a^* - redness; negative a^* - greenness)

b^* = yellow-blue colour. (Positive b^* - yellowness; negative b^* - blueness)

Vitamin C

Determination of vitamin C content was done by titration of the samples with 2, 6 dichlorophenol indophenol (Onwuka, 2005).

Carotenoid

The carotenoid content of the samples was measured by using UV Spectrometry (Khoo et. al, 2008). The wavelength at 450 nm was used to measure the optical density of the extract against a blank (3% acetone in petroleum ether) and the reading was compared with

the standard curve and the number of carotenoids was calculated as the given formula:

$$\text{Carotenoids (mg/100g)} = \frac{\text{concentration} \times \text{final volume} \times \text{dilution}}{\text{weight of sample}} \times 100$$

Determination of total, reducing, and non-reducing sugar (%)

Total and reducing sugars were estimated by the volumetric method (Bernetti et al., 2001; Kahraman et al., 2010). The percentage of reducing and total sugars were calculated by using the following formula-

$$\% \text{ Reducing Sugar} = \frac{\text{Fehlings Factor} \times \text{Dilution} \times 100}{\text{weight of sample} \times \text{titre}}$$

$\% \text{ Total Sugar} =$

$$\frac{\text{Fehlings factor} \times \text{volume made up} \times \text{dilution} \times 100}{\text{weight of sample} \times \text{sample taken from volume} \times \text{titre}}$$

Sensory evaluation

Banana samples T₁, T₂, T₃, T₄, T₅, and T₆ were subjected to sensory evaluation. A panel of 15 tasters evaluated mouthfeel, flavour, appearance and overall acceptability of the control banana sample and five supplemented banana samples. The effect of different treatments on sensory attributes was measured using a 9-scale Hedonic Rating Test.

Statistical analysis

All the experiments were performed in triplicates. One-way Analysis of Variance (ANOVA) was performed with a $p < 0.01$ level of significance. All the results were expressed as mean \pm SD (Standard Deviation of the mean). Regression analysis was performed to measure the correlation between physicochemical properties and heavy metals. Fisher's multiple range test was done to determine the most suitable treatment with the highest acceptability in all sensory attributes.

Results and Discussion

Effect of different treatments on ripening time of bananas

The artificial ripening process makes ripening faster and results in a variation in fruit's chemical properties. As shown in figure 1, the calcium carbide-induced bananas took less time to ripen than the control samples.

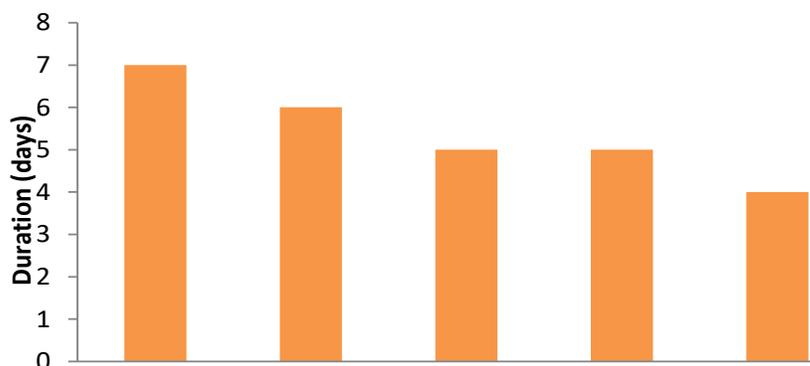


Figure 1. Effect of different treatments on ripening time of bananas Note: T₁ = Control sample; T₂ = Dipping in 1% CaC₂ solution; T₃ = Dipping in 2% CaC₂ solution; T₄ = Placing 5 g CaC₂ powder; T₅ = Placing 10 g CaC₂ powder; T₆ = Market sample.

It could be seen that the samples T₅ and T₆ took only 4 days to ripen which is less than the control sample. The sample T₁ followed the control sample closely as it took around 6 days to ripen. When CaC₂ reacts with water, acetylene gas was produced which was analogous to ethylene and the ripening process was accelerated (Lakade et al., 2018). Many of the fruits which we eat were ripened through CaC₂ to achieve faster ripening with uniform characteristics (Siddiqui & Dhua, 2008).

Heavy metal residues in calcium carbide ripened bananas

Artificial ripening process, such as using CaC₂ as a ripening agent contains heavy metals like arsenic and calcium as well as phosphorous; these possess a great risk to human health (Islam et al., 2018). The residual amounts of heavy metal contents in bananas are presented in Table 1.

Table 1. Effect of different carbide treatments on the heavy metal residue of ripened bananas

Treatments	Residual Arsenic (ppm)	Residual Calcium (mg/100g)	Residual Phosphorous (ppm)
T ₁	0.00095±0.0003 ^A	5.2±0.00 ^A	110.78±0.610 ^A
T ₂	0.0335±0.00031 ^B	6.817±0.080 ^B	119.270±0.292 ^B
T ₃	0.051±0.00208 ^C	7.323±0.137 ^C	123.917±0.204 ^C
T ₄	0.0418±0.0008 ^D	6.471±0.065 ^D	121.733±0.351 ^D
T ₅	0.0558±0.0005 ^E	6.915±0.009 ^B	124.303±0.075 ^C
T ₆	0.0627±0.00021 ^F	7.394±0.156 ^C	128.893±0.386 ^E

Note: T₁ = Control sample; T₂ = Dipping in 1% CaC₂ solution; T₃ = Dipping in 2% CaC₂ solution; T₄ = Placing 5 g CaC₂ powder; T₅ = Placing 10 g CaC₂ powder; T₆ = Market sample. Results in the same column with the same letter did not present any difference at the 0.05% significance level and different letters present significant differences at the 0.05% level.

For CaC₂-induced bananas, the value of arsenic residues by different treatments varied from 0.0335±0.00031 to 0.0627±0.00021 ppm. The control sample showed a negligible amount of arsenic residue (0.00095±0.00003 ppm) which could be due to soil conditions. The maximum value of arsenic residues was recorded in the market sample (0.0627±0.00021ppm). It indicated the possibility of using a high concentration of CaC₂ by the traders as a previous study showed that the market fruits ripened with CaC₂ contained high levels of arsenic (0.021-0.097 mg/kg) (Hakim et al., 2012). Calcium in bananas is influenced by the applied CaC₂ concentration. The high level of calcium in bananas could be due to the use of CaC₂ (Maduwanthi & Marapana, 2019). The highest amount of residual calcium, 7.394±0.156 mg/100g, was found to be in the market sample (T₆) which is significantly indifferent

from sample T₃ (7.323±0.137 mg/100g). The control sample (T₁) contained a relatively lower amount of calcium which was 5.2±0.00mg/100g. For bananas treated with CaC₂ concentration, calcium content was higher compared to the samples treated with powder CaC₂. It could be speculated that for the dipping condition, calcium penetrated in as well as deposited on the surface of the banana samples. Besides, calcium is known to be slightly soluble in water so it could have gone inside the fruit. The value of calcium found in bananas ripened without using calcium carbide could represent the actual amount of calcium present in banana fruits. For phosphorous, the amounts were found to be gradually increasing with the carbide concentration. The control sample showed the presence of phosphorous around 110.78±0.610 ppm which was lower than the treated samples. The highest

residues (128.893 ± 0.386 ppm) were found in sample T₆. The results showed the possibility of the traders using a very high concentration of CaC₂ to accelerate the ripening of bananas as CaC₂-induced fruits can have high phosphorous content (Hakim et al., 2012; Alam et al., 2001).

Physicochemical properties of ripened bananas with different treatments

The physicochemical properties of all the treated banana samples are presented in Table 2.

Table 2. Effect of different carbide treatments on the physicochemical properties of ripened bananas

Properties	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆
Moisture (%)	60.91±0.155 ^A	61.35±0.130 ^B	62.13±0.013 ^{CD}	61.263±0.035 ^B	61.952±0.005 ^C	62.33±0.019 ^D
p ^H	6.45±0.000 ^{ABC}	6.42±0.036 ^{ADE}	5.95±0.035 ^G	6.413±0.014 ^{BD^F}	6.13±0.030 ^H	6.43±0.004 ^{CE^F}
TSS(^o B)	23.00±0.040 ^A	19.00±0.009 ^B	20.01±0.019 ^C	21.00±0.00 ^D	25.00±0.00 ^E	17.03±0.036 ^F
Titration Acidity (%)	0.126±0.003 ^{AB}	0.119±0.004 ^{AC}	0.099±0.010 ^{DE}	0.116±0.001 ^{BC}	0.103±0.002 ^{DF}	0.098±0.00 ^{EF}
Total sugar (%)	17.34±0.129 ^F	16.35±0.031 ^{ABC}	16.74±0.069 ^G	16.33±0.016 ^{ADE}	16.17±0.105 ^{BD}	16.41±0.002 ^{CE}
Reducing sugar (%)	5.59±0.005 ^{AB}	5.59±0.002 ^{AC}	4.985±0.004 ^D	5.596±0.002 ^{BC}	5.152±0.002 ^E	4.957±0.005 ^F
Vitamin C (g/100g)	27.03±0.252 ^A	23.00±0.200 ^B	19.002±0.093 ^C	20.04±0.047 ^D	17.00±0.012 ^E	16±0.024 ^F
Carotenoid (µg/100g)	47.03±0.153 ^A	51.00±0.000 ^B	53.033±0.043 ^C	52.03±0.058 ^D	57.044±0.041 ^E	43±0.033 ^E

Note: T₁= Control sample; T₂= Dipping in 1% CaC₂ solution; T₃= Dipping in 2% CaC₂ solution; T₄= Placing 5 g CaC₂ powder; T₅= Placing 10 g CaC₂ powder; T₆= Market sample. Results in the same row with the same letter did not present any difference at the 0.05% significance level and different letters present significant differences at the 0.05% level.

In the present study, moisture content for different treatments was found to exhibit no significant difference ($p < 0.01$), for example, samples T₂ and T₄ were insignificant in terms of change in moisture percentages as well as samples T₃ and T₅. The sample T₃ also showed no significant difference from the market sample. For pH, no definite pattern was found in terms of increase or decrease in values. The same could be observed for total sugar, reducing sugar and TSS. In contrast to that, for vitamin C, it could be seen that the application of CaC₂ decreased the amount of vitamin C. Though for carotenoid content, the scenario was the total opposite as the amount increased in treated samples. These could happen as previous studies stated a decrease in protein content and an increase in moisture and mineral contents in CaC₂ ripened fruits (Gunasekara et al., 2015). As the concentrations of pH, titration acidity, and vitamin C were found to be decreasing with CaC₂ treatments, it could dangerously affect the fruit quality and possess risks to health (Sogo-Temi et al., 2014).

Correlation of physicochemical properties and heavy metal contents of carbide-treated bananas

Regression analysis was done to assess the relationship between physicochemical properties and residual heavy metals. Correlation coefficients were used to determine the linear relationship between two variables. The values always ranged between -1 and +1, where -1 pointed to a strong negative relationship and +1 indicated a positive relationship. The correlation between the residual heavy metals and physicochemical properties is presented in Table 3. The results of the correlation showed that arsenic, calcium and phosphorous were negatively correlated with the pH values ($R = -0.4363$, -0.337 , and -0.497 respectively).

Table 4. Effect of different carbide treatments on the color of ripened bananas

As Pearson correlation coefficient, the range of R from -0.40 to -0.96 referred strong negative relationship. The titration acidity was positively correlated with all heavy metal content with significance at a 5% level, which meant that the titration acidity increased with increasing heavy metal content.

Table 3. Correlation coefficient among physicochemical properties and heavy metal contents of carbide-treated bananas

Properties	Arsenic	Phosphorus	Calcium
p ^H	-0.436	-0.337	-0.497
Total sugar	-0.784**	-0.757**	-0.69*
Titration acidity	0.541*	0.595*	0.651*
TSS	-0.322	-0.441	-0.502*

Note: **Significant at 1 percent level. *Significant at 5 percent level

Effect of different carbide treatments on the colour of ripened bananas

The colour is an important quality attribute from the consumer's point of view. The colour difference is shown in Table 4. From the table, it could be observed that the L* value (lightness) decreased with increasing CaC₂ from 77.775 to 66.235. The value of a* (indicates greenness) increased with the addition of CaC₂ from -1.171 to 0.04. The highest greenness was marked in bananas treated with 5g CaC₂ and b* (indicates yellowness) is decreasing with increasing CaC₂ from 20.445 to 18.695. The maximum yellowness was recorded in the Control sample. The total colour difference ΔE was highest in the control sample (77.822), and the treated samples mostly showed minimal difference in ΔE from the market sample. So, it could be said that chemically ripened fruit would show a yellow or ripened appearance than the naturally ripened one externally, but the tissue inside would not ripen or itself remains raw (Rahman et al., 2008).

No. of treatments	Treatments (T)	L*	a*	b*	ΔE
T ₁	Control sample	77.775 ^A	-1.171 ^A	20.445 ^A	77.822 ^A
T ₂	Dipping in 1% CaC ₂ solution	73 ^B	-0.865 ^B	20.145 ^A	75.733 ^B
T ₃	Dipping in 2% CaC ₂ solution	66.235 ^C	-0.055 ^C	18.695 ^B	68.822 ^C
T ₄	Placing 5g CaC ₂ powder	72.57 ^D	0.04 ^D	19.38 ^C	75.113 ^B
T ₅	Placing 10g CaC ₂ powder	72.23 ^E	-0.535 ^E	18.905 ^B	74.665 ^B
T ₆	Market sample	73 ^B	-0.865 ^B	19.33 ^C	75.521 ^B

Note: T₁ = Control sample; T₂ = Dipping in 1% CaC₂ solution; T₃ = Dipping in 2% CaC₂ solution; T₄ = Placing 5 g CaC₂ powder; T₅ = Placing 10 g CaC₂ powder; T₆ = Market sample. Results in the same column with the same letter did not present any difference at the 0.05% significance level and different letters present significant differences at the 0.05% level.

Sensory properties of the banana samples

Artificial ripening agents may help to increase the ripening process but affects the nutritional and sensory quality (Hossain et al., 2015). Fruits ripened through artificial ripening agents have an overly soft texture and are inferior in taste and flavour, compared to the naturally ripened ones (Nura et al., 2018). The sensory properties of bananas are given in figure 2.

From figure 2, it could be observed that sensory properties declined with the increase in carbide concentration. The application of CaC₂ not only harms the business-based qualities of fruits but also greatly affects the physicochemical, nutritional and antioxidant properties of fruits (Mahmood et al., 2013). In figure 2 the mean scores for mouthfeel, flavour, appearance and overall acceptability preferences are presented. One-way analysis of variance (ANOVA) on sensory qualities of banana samples was performed and the results showed that there was a significant (p<0.05)

difference in mouthfeel acceptability among the bananas. Fisher’s multiple range test indicates that the mouthfeel of sample T₁ was most acceptable by the tasters as it achieved better mouthfeel acceptability scores than other samples. Similar to the mouthfeel, flavour preferences also showed a significant (p<0.05) difference in flavour acceptability. Fisher’s multiple ranges showed that the flavour of sample T₁ was preferred and sample T₆ was least preferred relative to another sample at a 5% level of significance. There was a significant appearance difference (<0.05) between the control and other samples. The Fisher’s multiple range test for appearance preference was conducted and the findings showed that the control sample was more acceptable and significantly different from other samples. For the overall acceptability, Fisher’s multiple range test showed that each sample was significantly different from one another at a 5% level of significance. So, Sample T₁ is the most preferable relative to others.

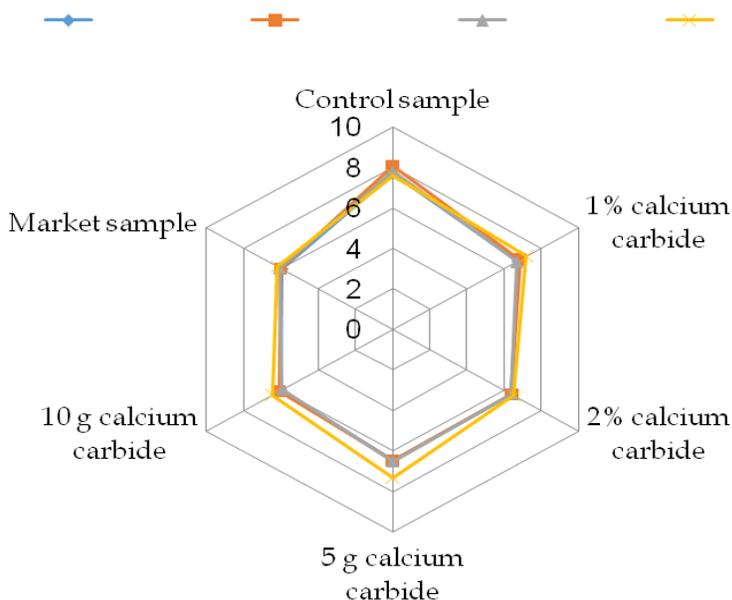


Figure 2. Effect of different treatments on Mouthfeel, Flavour, Appearance, Overall acceptability of bananas

Conclusion

The detection of risk assessment in fruits is important for researchers, consumers, industries, and regulatory agencies. The present study has provided the level of health risk associated with calcium carbide-induced bananas. The study showed a high level of heavy metal content in the market sample compared to the naturally ripened and treated samples. Metal concentrations increased with increasing calcium carbide concentration. This study also indicated moisture content increased with the increase in calcium carbide concentration while the other physicochemical properties decreased with increasing treatment. The physicochemical properties other than titrable acidity negatively correlated to arsenic, phosphorus and calcium, which mean that the titrable acidity increased with increasing heavy metal content. The sensory properties of bananas were also decreasing with an increase in CaC_2 concentration. This represents the nutritional quality of bananas was reduced when bananas ripened with calcium carbide. It also indicated a high health risk. This study recommends the natural sample over the treated or market sample because it presented lower heavy metal content, better sensory profile and lower exposure on daily intake compared to the treated and market samples. It is high time to take proper regulatory actions to control the uses of CaC_2 in ripening bananas. Therefore, the challenge is to develop an effective and efficient method for safe ripening and removal of calcium carbide residues in traditionally ripened bananas. In this study, a new idea will be grown in all people about the natural and artificial ripened banana.

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