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The Antioxidant Potential and Vitamin C Content in Indian Dessert Bananas

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Abstract: Background: While many banana types that are loved by locals for their unique flavor and health advantages have their roots in India, not all of these kinds have been thoroughly analyzed for their nutritional content. A vitamin C deficit affects over 75% of the Indian population, despite the fact that the majority of the recommended daily allowance comes from fruits and vegetables. Approach: The vitamin C (AA) concentration in the pulp of three local banana types was examined using the standard AOAC dye titration technique at various ripening stages. Using spectrophotometry and a DPPH radical scavenging experiment, we determined the AOX potential of freshly harvested pulp at various ripening stages. The results showed that varieties Cavendish, RB, and Nendran, which are genotype AAA, had substantially lower AA content at 10, 0.3, and 0.2 mg/100gm FW, respectively, compared to varieties NRB and EB, which are genotypically composed of the AAB genome, which had the highest AA content at climacteric (edible ripe) stages with 45 and 36 mg/100g FW, respectively. In the edible ripe stage of NRB, the AOX potential of fresh pulp was 94% per gram, whereas in EB, the post ripe phases showed 77.5% and 73%, respectively. RB had 30% AOX activity per g FW, whereas Nendran exhibited 60%. The results of this study provide important information for the selection of vitamin C-rich banana varieties for cultivation in order to combat the vitamin C deficiency that affects many tropical countries. Bananas are popular, easy to grow, and eaten fresh all year round, so this study marks a first.

Keywords: Fruit, Antioxidant, Banana, Indian Dessert, Vitamin C

Introduction

Vitamin C is a class of compounds that are structurally similar to ascorbic acid but are soluble in water and cannot be produced or retained by the human body. Consequently, you should replenish your vitamin stores every day by eating enough of fruits and vegetables. According to Figueroa-Méndez and Rivas-Arancibia (2015), this vitamin is essential for a wide range of metabolic processes, including enzyme co-substrates, redox state maintenance, neurophysiological functions (such as collagen synthesis), wound healing, and the prevention and treatment of chronic degenerative diseases. According to Richelle et al. (2006), the adrenal glands, brain, liver, and skeletal muscles have the largest concentrations of ascorbate in the human body, with concentrations of 550 mg/kg, 140 mg/kg, 125 mg/kg, and 35 mg/kg, respectively. This highlights the significant role of this vitamin. When taken as a whole, AA's metabolic requirements and powerful antioxidant properties are thought to provide humans with enormous health advantages.

Many tropical and less developed nations suffer from severe deficiency (75%), in contrast to a few affluent nations whose people suffer from mild deficiency (10-18%) (Ravindran et al 2011). It is unusual to eat fresh, uncooked veggies, even though India is the world's leading producer of AA-rich fruits and vegetables. The widespread use of pressure cooking also reduces the bioavailability or completely destroys vitamin C. Indian gooseberry (Emblica officinalis Linn.), guava, and oranges are among the fruits that are rich in

vitamin C sources that range from 200 to 400 mg/100 g (Raghu et al., 2007), however they are only available during a certain season and aren't affordable for everyone. The fact that not everyone like such acidic fruits is crucial. On the other hand, bananas and other inexpensive fruits are eaten all year round and are an essential component of many traditional ceremonies. Even if there is only 7 to 10 mg/100 g FW, the most widely grown banana variety, Cavendish (Musa acuminata, with genome AAA), is still a decent source (www.naturalhub.com). Although several dessert banana types originate in India, only few have had their vitamin C content studied (Vasanthkumar et al., 2013; Venkatachalam et al., 2008). Thus, the purpose of this research was to examine widely-grown banana fruit kinds in the area and determine when each variety's AA content is at its peak. The types that were chosen, NRB, EB, and RB, are nutrient dense (Lokesh et al., 2014), with RB having the greatest concentration of carotenoids (20µg/g FW). Due to the lack of reported adverse responses, a kind of banana known as "silk" is considered the best NRB for newborns to eat when they are weaning. According to the Geographical Indications Journal published by the Government of India (8-11): 44-49 in 2005, this particular variety is also recognized as a geographical indicator in India and is marketed under a brand name.Vitamin C concentration, which is responsible for the NRB plant's antioxidant activity, has not been recorded, despite a few studies reporting significant antioxidant levels in various portions of the plant. This is why we set out to measure the vitamin C content and anti-oxidative potential of a few healthy banana types throughout their ripening processes in the current research.Materials and Methods

All chemicals used were of analytical grade obtained from Sigma Aldrich and Hi-Media Chemicals, Mumbai. Glass triple distilled water and sterilized glass-wares were used throughout. Materials were handled with latex gloved hands. Banana fruits of different ripening stages were selected as reported earlier (Lokesh et al., 2014). The AOAC procedure No. 967.21 (2005) recommended forroutine food and vitamin formulations analysis was used toconfirm its suitability by analyzing and comparing with a vastly studied banana variety, i.e., Cavendish. This AOAC method has also been recommended for vitamin Cquantification with satisfactory accuracy in several advanced institutions (Anonymous, 2011). Use of meta- phosphoric acid-acetic acid extraction

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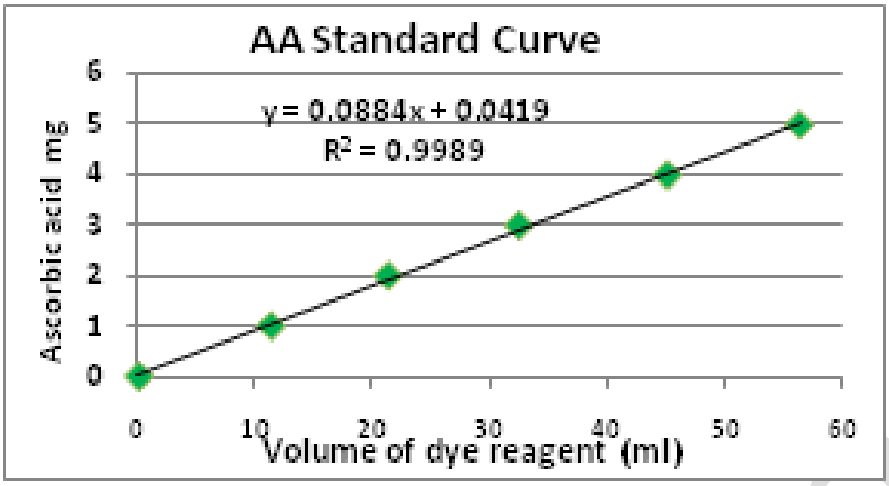
solution has been reported to efficiently extract 99% of ascorbic acid from fruit samples (Hernandez et al., 2006). The principle behind this method is based on the reduction of oxidation- reduction indicator dye, 2, 6-dichloroindophenol, which is blue at alkaline pH, colourless at neutral and pink in acidicpH. The blue dye is reduced to a colourless solution by ascorbic acid and when the acidic pH is reached a stable pink colour is obtained when traces of dye remains inacidic condition heralding the end point reaction.



Various concentrations of standard AA prepared in thesame meta-phosphoric acid-acetic acid extraction solution was analysed to construct the standard curve. These resultswere verified by sample spiking, which also ascertained reported values for vastly studied Cavendish banana, AA standard as well as banana samples spiked with AA. Although this method does not yield DHAA levels in samples, the method was adopted due to its ease and rapidity of analysis.

Banana fruits of 4 distinctly different ripening stages were selected for analyses. One gram of fruit pulp (in triplicate) from each variety was crushed using mortar and pestle in an aliquot of extraction solution and the sample was completely recovered from mortar-pestle to make the final volume 10 ml collected in capped-centrifuge tubes. All such samples were centrifuged at 10000 rpm for 10 min at 4°C and the supernatant was used for estimation. Titration was repeated thrice for each independently extracted sample as well as spiked samples (with known aliquot of AA standard solution) and quantified as suggested in the AOAC procedure No. 967.21.

Determination of free-radical scavenging activity: While there are several methods to determine radical scavenging efficacy, the present study chose to analyzethis by using the vastly accepted DPPH radical scavenging by photometric method, which is simple, rapid and reasonably accurate without the requirement of expensive equipment facility and special skills. The precision of this method has also been verified and confirmed through a collaborative study by a group of laboratories involving many countries (Plank et al., 2012). DPPH· reagent solution was prepared by using 10 mg/250 ml (0.004%) byfirst dissolving in 100 ml of HPLC grade methanol95% (intriple distilled water) in a flask wrapped with aluminium foil to protect from light. The compound was allowed to dissolve by adding a magnetic bar and allowing stirring for30 min. To this 150 ml of 95% ethanol was added and allowed to stir for another 10 min.

Pulp from banana fruit of different ripening stages were chosen for the study. Different sample quantities ranging from 200 mg to 1000mg were separately taken in 2 ml Eppendorf tubes and crushed using round-edged glass rod in 1 ml of triple distilled water. The capped tubes were centrifuged at 10000rpm for 15 min at 4°C. The supernatant was individually analyzed by adding DPPH solution and incubating on a wrist shaker for 20 min, followed by radical scavenging assay by recording change (decrease) in absorbance at 517nm read against reagent blank by double beam spectrophotometer.

Experiment was repeated with three separate extractions and the average of three readings was plotted. Pure L- ascorbic acid (AA) at different concentrations prepared in triple distilled water was also analyzed similarly and used as a comparative standard.

1. Results and Discussion

Results obtained for different concentrations of AA by the dye titration method (Figure 1) were linear with a regression value of 0.996. This data is almost similar to that reported on the basis of spectrophotometric method (Kapur et al., 2012; Al-Majidi and Al-Qubury, 2016); indicating the method is acceptable and the values are in the comparable range as in other studies. This

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is further supported by the AA concentration obtained for vastlyreported Cavendish banana, which is 10±2mg/100g (USDA database of 2018, Terrago-Trani et al., 2012), withonly one exceptional report, where 19mg/100g wasobserved (Abdulrazak et al., 2015). Therefore, it is implicitthat the results observed for the varieties of banana under consideration in this study are grossly acceptable.

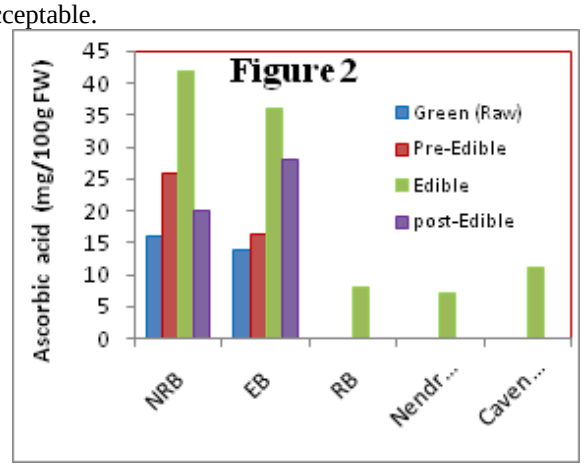

Figure 2: Concentrations (mg/100g FW) of AA in different banana varieties. Data presented as an average of3 replicates

The concentration of AA in 100g of NRB pulp (Figure 2) was quite high ranging from 16 mg in unripe green stage, 25 mg in semi ripe reaching an average highest level of 42 mg at edible ripe stage, declining to 20mg at post edible ripe stage. This is the highest content of vitamin C reported so far among banana varieties. The second highest content with a similar trend during ripening was observed in EB with 14 mg in green unripe stage, increasing to 22mg in pre-edible ripe stage reaching a highlevel of 32mg at edible ripe stage which declined in post- ripened stage to 27 mg. The decline however was lesser than that of NRB at this stage of ripening. Other twovarieties, Nendran and red banana recorded very low levels of about 7 to 8 mg/100g pulp only at edible ripe stage. The content of AA in vastly studied Cavendish at edible ripe stage was about 11 mg/100g pulp as reported invarious studies. Here it is worth mentioning that the biochemical profile, particularly the AA content, is reflected in their genetic relationship. An earlier study where different south Indian dessert banana varieties and their genetic relationships were analysed by DNA finger- printing markers such as RAPD and ISSR (Venkatachalamet al., 2008), a close relationship between varieties NRB and EB has been established and both have similar genomic composition of AAB. This is true for other bananas such as RB and the Cavendish (with genome AAA) displaying almost similar low content of AA.

DPPH radical scavenging activity: Pure AA compound rendered over 96% DPPH radical scavenging at1000µg/ml (1 ppm) indicating that a low level of200µg/ml could bring about 50% inhibition(IC_{50}=200µg/ml) under in vitro conditions, as in other studies (Divya et al., 2012). However, an earlier studywhere different standard antioxidant compounds were analyzed by DPPH radical scavenging assay documented the requirement of 10ppm of AA for 96% radicalinhibition and only 17.4% inhibition was obtained for 1 ppm of AA (Veigas et al., 2007). In the present study, NRB pulp imparted almost 100% inhibition at 1g/ml, where the lower levels of pulp respectively showed lower levels of inhibition (Figure 3A). Surprisingly, 200 mg and 400 mg of NRB pulp at post ripening stage had slightly higher radical scavenging efficacy than that of AA (Figure 3A). The radical scavenging efficacy of EB (Figure 3B)was much lower (average IC_{50} value of 800mg/ml) than that of edible ripe stage of NRB, although significantly higher than the values observed for RB and Nendran (Figure 3C).

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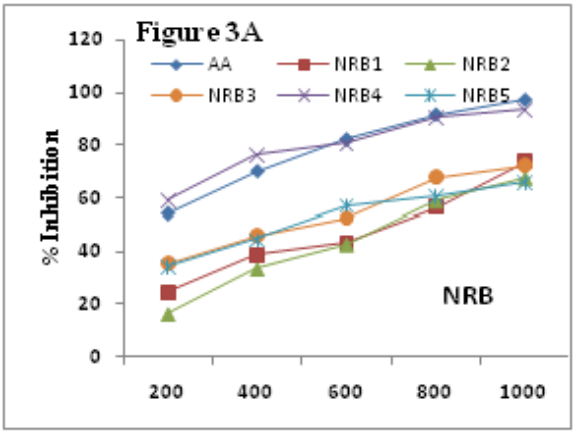

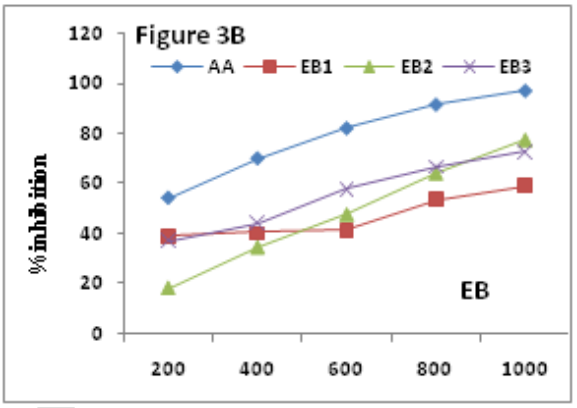

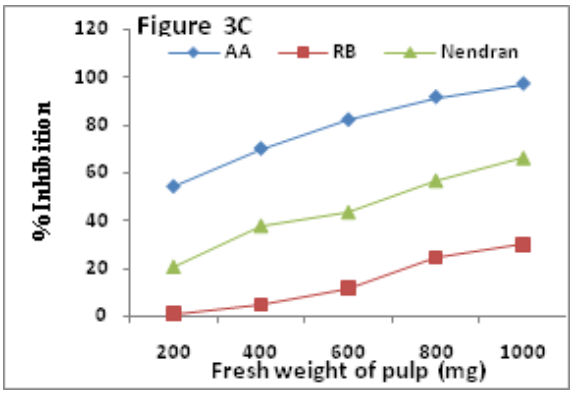

Figure 3: Radical scavenging activity of AA (µg/ml) andbanana pulp at different ripening stages

Despite the fact that both RB and Nendran have rich content of carotenoids such as alpha-carotene and beta- carotene (Lokesh et al., 2014) that are well-documented antioxidants (Neelwarne and Veigas, 2012; Divya et al., 2012), the pulp of Nendran recorded 66% inhibition at highest concentration of 1000mg/ml whereas at similarquantity of pulp extract of RB showed only 30% inhibition. These results clearly indicate that the antioxidant efficacy in terms of radical scavenging is directly and mainly related to the content of AA in the pulp. In a study which compared the antioxidant potential of the banana extract with that of the sample in *in vitro* gastro-intestinal simulation model, a higher efficacy was observed in the physiological enzyme (Bhatt and Patel,

2015) indicating that much higher efficacy may be availed by consuming NRB fruits on a daily basis.

2. Conclusion

The present study for the first time has evaluated vitamin C content and the antioxidant potential in some Indianlocal varieties of bananas and found that they actuallysynthesize and store much higher levels of this vitaminthan the widely cultivated Cavendish type. Each NRB or RBA banana having at least 200g of pulp is sufficient to provide the RDA of vitamin C. Assuming

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that in addition to the estimated level of AA, an equal amount of DHAA (additional) could be present in many fruits and vegetables(Kiuchi et al., 2017), the actual bio-availability of vitamin C from NRB could be much higher. The antioxidant potential is also highest in NRB compared to other bananas. Owing to these health benefits, the varieties NRBand EB hold high promise in addressing vitamin C deficiencies in tropical countries where intense cooking is practiced whereas bananas are consumed fresh(uncooked). Popularizing these varieties and their regular cultivation coupled with controlled processing and development of newer products are helpful for planning nutritious diets.

Author Contribution

The study was conceived and designed by BN. AAB and CKP performed the experiments and recorded data, VBR contributed to the sample collection, spectrophotometric analyses and discussion, and BN analyzed data wrote the manuscript.

Conflict of Interest

All authors have declared no conflict of interests.

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