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Vitamin C Content in Indian Dessert Bananas and their Antioxidant Potential

Abhishek AB[1], Chandankumar KP[1], Vinay B Raghavendra[2], Bhagyalakshmi Neelwarne[1, 3*]

[1]Neelgen Greentech, Vinayakanagar, Mysore 570012, India
[2]PG Department of Biotechnology, Teresian College, Siddharthanagar, Mysore 570011, India
[3]Formerly - Plant Cell Biotechnology Department, CSIR-Central Food Technological Research Institute, Mysore 570020, India

*__Corresponding author__: bneelwarne[at]gmail.com

Abbreviations: ***AA: L-ascorbic acid, AOX: antioxidant, DHAA: Dehydroxy ascorbic acid, DPPH: 2, 2-diphenyl-1-picrylhydrazyl, EB: Elakki banana, FW: Fresh weight, NRB: Nanjanagudu rasabale banana, RB: Red banana, RDA: Required daily allowance.***

Abstract: ***Background:*** ***Although India is a place of origin of several banana varieties that are locally popular for their exotic taste and health benefits, some of these varieties have never been subjected to comprehensive nutritional analysis. Fresh fruits and vegetables are the sources of daily requirement of vitamin C and 75% of Indian populations often suffer from this deficiency.*** ***Method:*** ***Vitamin C (AA) content in fruit pulp of three local varieties of banana at different ripening stages was analyzed by standard AOAC dye titration method. The AOX potential of fresh pulp from different ripening stages was evaluated by DPPH radical scavenging assay by spectrophotometry.*** ***Results:*** ***The highest content of AA was observed in climacteric (edible ripe) stages in varieties NRB (45mg/100g FW) and EB (36 mg/100g FW), both are genotypically composed of AAB genome whereas varieties Cavendish, RB and Nendran of genotype AAA showed much lower AA content of respectively 10, 0.3 and 0.2 mg/100gm FW. The AOX potential of fresh pulp was 94% per gram in the edible ripe stage of NRB whereas in EB the post ripe stages exhibited 77.5% and 73%. RB and Nendran showed AOX activity of 30% and 60% per g FW.*** ***Conclusion:*** ***This study has for the first time identified banana varieties rich in vitamin C and since bananas are consumed fresh, liked by many and produced year-round, the present information is helpful in choosing such varieties for cultivation to address vitamin C deficiency prevailing in many tropical countries.***

Keywords: Vitamin C, Indian Dessert, Banana, Antioxidant

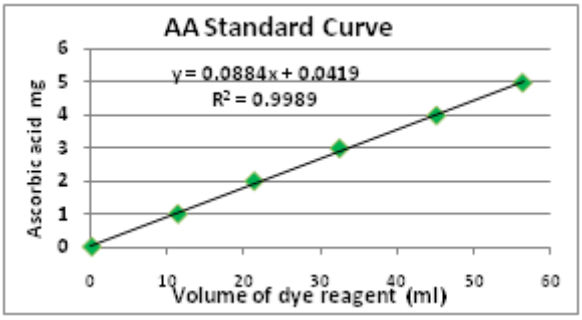
1. Introduction

Vitamin C is a group of analogues of ascorbic acid, highly soluble in water and neither synthesized or stored in humans. Therefore, this vitamin needs to be restored daily through diet – mainly from fruits and vegetables. This vitamin plays important roles in various metabolic processes in human body from acting asco-substrate for many enzymes, maintenance of redoxto neurophysiological functions, including collagen synthesis, wound healing and many positive effects on chronic degenerative diseases (Figueroa-Méndez and Rivas-Arancibia, 2015). Within human body, the highest concentrations in the form of ascorbate are found in adrenal glands (550 mg/kg), brain (140 mg/kg), liver (125 mg/kg) and in skeletal muscles with a concentration of 35 mg/kg (Richelle et al., 2006) indicating the high importance of this vitamin. The dual function of AA as a metabolically needed vitamin as well as imparting strong antioxidant functions, when considered together, is assumed to impart tremendous health benefits to humans.

While populations of several developed countries have minor vitamin C deficiencies (10- 18%), many tropical and less developed countries reel under severe deficiency (75%) (Ravindran et al 2011). For instance, though a country like India is the highest producer of fruits and vegetables that are rich in AA, consuming fresh (uncooked) vegetables is uncommon. Added to this, pressure cooking is prevalent due to which vitamin C is either destroyed or results in low bioavailability of this vitamin. Although certain fruits such as oranges, guava and Indian gooseberry (*Emblica officinalis* Linn.) are rich sources of vitamin C ranging from 200 to 400mg/100g (Raghu et al., 2007), their availability is restricted to a particular season and their inaccessibility to every strata of population is a concern. Above all such astringent fruits are not liked by all. Contrarily, certain fruits such as bananas are cheap, consumed throughout the year and are an integral part of many traditional rituals. However, the vastly cultivated variety of banana being Cavendish (*Musa acuminata* - having genome AAA) has as little as 7 to 10 mg/100g fresh weight (FW), which is still considered as a good source (www.naturalhub.com). India is a place of origin of many varieties of dessert bananas (Venkatachalam et al, 2008) of which only a few have been analysed for vitamin C content (Vasanthkumar et al, 2013). Therefore, the present study was done with an objective of analyzing popular local varieties of banana fruit and to find out the stage of ripening at which AA is highest in each variety. Among the selected varieties, NRB, EB and RB are rich in nutrients (Lokesh et al., 2014) with highest content of carotenoids (20µg/g FW) in RB. The tastiest NRB is a type of 'silk' banana traditionally used as weaning food for infants since no allergic reactions have been observed so far. This variety also enjoys a geographical indicator status in India and sold as a branded item (Geographical Indications Journal, Govt. of India (8-11): 44-49, 2005).Although a few studies reported high levels of antioxidants in different parts of NRB plant, the content of vitamin C which chiefly contributes to its antioxidant property has not been reported. For all these reasons, the present study was conducted to estimate vitamin C content and anti-oxidative potential of selected nutritious banana varieties at different ripening stages.

2. 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 for routine food and vitamin formulations analysis was used to confirm its suitability by analyzing and comparing with a vastly studied banana variety, i.e., Cavendish. This AOAC method has also been recommended for vitamin C quantification with satisfactory accuracy in several advanced institutions (Anonymous, 2011). Use of meta-phosphoric acid-acetic acid extraction 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 acidic pH. 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 in acidic condition heralding the end point reaction.



Various concentrations of standard AA prepared in the same meta-phosphoric acid-acetic acid extraction solution was analysed to construct the standard curve. These results were 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 analyze this 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%) by first dissolving in 100 ml of HPLC grade methanol95% (in triple 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 for 30 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.

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

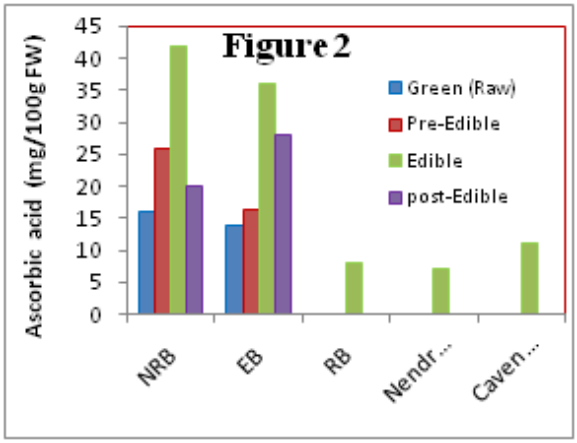


Figure 2: Concentrations (mg/100g FW) of AA in different banana varieties. Data presented as an average of 3 replicates

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 high level 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 two varieties, 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 in various 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 (Venkatachalam et 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 at 1000µg/ml (1 ppm) indicating that a low level of 200µg/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 study where different standard antioxidant compounds were analyzed by DPPH radical scavenging assay documented the requirement of 10ppm of AA for 96% radical inhibition 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).

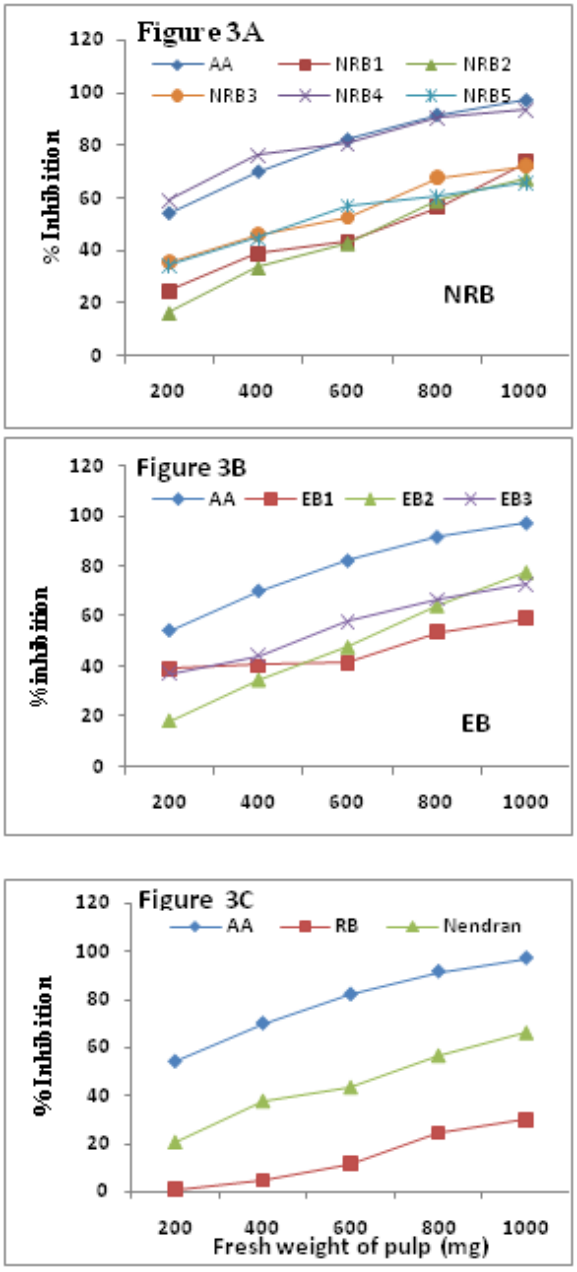


Figure 3: Radical scavenging activity of AA (µg/ml) and banana pulp at different ripening stages

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 similar quantity 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.

4. Conclusion

The present study for the first time has evaluated vitamin C content and the antioxidant potential in some Indian local varieties of bananas and found that they actually synthesize and store much higher levels of this vitamin than 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 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 NRB and 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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