



**DETERMINATION AND COMPARISON OF NON ENZYMATIC
ANTIOXIDANTS FROM DIFFERENT LOCAL VARIETIES OF BANANA
(MUSA SP.)**

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ABSTRACT

The present study mainly focused on quantification and comparison of non enzymatic antioxidants from pulp extracts of nine varieties of banana viz Kadali, Karpooravalli, Monthan, Nenthiran, Pachainadan, Poovan, Rasthali, Robusta and Sevvazhai. The non enzymatic antioxidants such as total reduced glutathione, vitamin A, vitamin C, vitamin E were quantified from banana pulp extract. The data obtained were subjected to statistical analysis and results were compared. All the banana varieties exhibited significant amount antioxidants. Rasthali, Poovan, karpooravalli Robusta showed highest amount of antioxidant enzymes compared to other varieties.

KEY WORDS: free radicals, nonenzymatic antioxidants, banana varieties, total reduced glutathione, vitamins.



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INTRODUCTION

Free radicals are reactive oxygen species derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants and industrial chemicals. In vivo, some of these ROS play positive roles in cell physiology, however they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and other DNA mutations, leading to cancer, degenerative, and other diseases [1]. Thus, there is an increased evidence for the participation of these free radicals in the aetiology of diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative disorders, ageing, etc. Mammalian cells possess elaborate defence mechanisms for radical detoxification. Antioxidants are agents, which scavenge the free radicals and prevent the damage caused by them. Some of these compounds are of exogenous nature and are obtained from food. Examples include antioxidants like α -tocopherol, β -carotene, and ascorbic acid, and some micronutrient elements such as zinc and selenium[2]. β -carotene is a member of the carotenoid family, a group of powerful antioxidants that also includes α -carotene, lycopene, zeaxanthin, and lutein. Hence, β -carotene has been shown to help guard against cancer and heart disease. Vitamin C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis [3]. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation[4]. Glutathione is body's master antioxidant and one of the most important cleansing and healing agents.

Glutathione blocks free radical damage and help to recycle Vitamins E and C, therefore plays a key role in their function. Melatonin is an antioxidant that can easily cross cell membranes and the blood-brain barrier[5]. Melatonin is a direct scavenger of OH, O_2^- , and NO[6]. The present study has been aimed to evaluate and compare non enzymatic antioxidant compounds from pulp extracts of nine local varieties of banana to assess its protective role against free radical induced cell damage.

MATERIALS AND METHODS

BANANA SAMPLE

The banana varieties viz. Kadali, Karpooravalli, Monthan, Nenthiran, Pachainadan, Poovan, Rasthali, Robusta and Sevvazhai., were collected locally from various places in Sathyamangalam (Tamil Nadu, India). The banana specimens were authenticated by Dr.T.N.Balamohan, Professor and Head, Faculty of Horticulture, Tamil Nadu Agricultural University, Coimbatore. A voucher specimen of the sample has been deposited in the herbarium of the department.

CHEMICALS

All the solvents and other chemicals which were used during this study were purchased from Merck and s.d. Fine-Chemicals, Mumbai.

METHODS

Estimation of Total Reduced *Glutathione*

Total reduced glutathione was estimated by the method of Moran *et al.*, 1979 [7]. 0.5 ml of 20% tissue homogenate was mixed with 0.5 ml of 5% TCA. The precipitate was removed by centrifugation. 0.1 ml of the supernatant was made up to 1.0ml with 0.4M Phosphate solution and 2.0 ml of the freshly prepared DTNB reagent was added. The absorbance was read after 10 minutes at 412nm against a reagent blank. A set of standards were also

treated in the above manner. The amount of glutathione was expressed as $\mu\text{g}/\text{mg}$ protein.

Estimation of Vitamin A

1ml of 10% homogenate was mixed with 1ml of 2N KOH in 90% alcohol. The mixture was refluxed for 20min at 60°C. Then saponified mixture was cooled to room temperature 25ml of water was added and mixed well and transferred to separating funnel. Extracted thrice with 25,15,10ml petroleum ether. Ether extract was pooled and washed with 50-100ml of distilled water repeatedly. Petroleum ether extract was dried by adding anhydrous sodium sulphate. The aliquots were evaporated to dryness at 60°C in a water bath. Residue taken immediately and 2.0ml of TFA reagent was added. The blue colour developed was read at 620nm.

Estimation of Vitamin C

Estimation of vitamin c was performed by the method of Sadasivam and Manickam, 1997[8] .5g of sample was ground in 25-50ml of 4% oxalic acid. The sample was centrifuged at 10000 rpm for 20 min.10ml aliquots were transferred to conical flask. A few drops of activated charcoal solution were added. The solution was made up to 25 ml with 4% oxalic acid.1 ml aliquots of the above solution were taken and 3ml of the distilled water was added. To the above solution, 1 ml of DNPH was added. This was incubated at 37°C for 3 hours.7ml of 80% H₂SO₄ (in ice) was added to the test tubes and they were

incubated at room temperature for 30 min. The absorbance was read at 540nm.

Estimation of Vitamin E Sample extraction :

1g of banana pulp was homogenized in a mortar and pestle and transferred to a conical flask. 50ml of 0.1N sulphuric acid was added slowly without shaking. Stoppered and allowed to stand overnight. The next day contents of the flask were shaken vigorously and filtered through whatmann.1 filter paper, discarding the initial 10-15 ml of the filtrate. Aliquots of the filtrate were used for the estimation. 1.5ml of pulp extract was pipetted into a Stoppered centrifuge tube, 1.5ml of the standard and 1.5ml of water respectively. To the test and blank added 1.5ml of ethanol and to the standard added 1.5ml of water. 1.5ml xylene was to all the tubes, stoppered, mixed well and centrifuged. Transferred 1.0ml of xylene layer into another stoppered tube, taking care not to include any ethanol or protein. Added 1.0ml 2,2' dipyridyl reagent to each tube, stoppered and mixed. Pipetted out 1.5ml of the mixtures into spectrophotometer cuvettes and read absorbance of test and standard against the blank at 460nm. Then in turn beginning with the blank, added 0.33ml of ferric chloride solution. Mixed well and after exactly 1.5minutes read test and standard against the blank at 520nm. The amount of vitamin E can be calculated using the formula.

$$\Delta A_{520\text{nm}} - \Delta A_{450\text{nm}} \times \text{conc}[S] \times \text{OD of std} \times \text{Total volume}$$

$$\text{Vitamin E } (\mu\text{g/g}) = \frac{\Delta A_{520\text{nm}} - \Delta A_{450\text{nm}} \times \text{conc}[S] \times \text{OD of std} \times \text{Total volume}}{\Delta A_{520\text{nm}} \times \text{Vol for experiment} \times \text{wt of sample}}$$

$$\Delta A_{520\text{nm}} \times \text{Vol for experiment} \times \text{wt of sample}$$

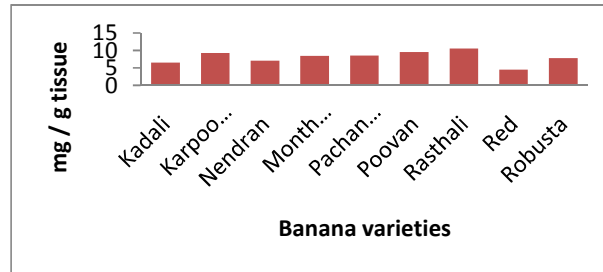
RESULTS AND DISCUSSION

Estimation of total reduced glutathione

Glutathione (GSH) is a tripeptide. It contains an unusual peptide linkage between the amine group of cysteine and the carboxyl group of the glutamate side chain. Glutathione, an antioxidant, helps protect cells from reactive oxygen species such as

free radicals and peroxides. High levels of glutathione appear to protect against the dangers of cancer, heart disease, premature aging, autoimmune diseases, damage from many pharmaceutical drugs, and chronic illnesses

Figure 1
Total reduced glutathione content in pulp extracts of nine varieties of banana.



Total reduced glutathione and vitamin A content in pulp extracts of nine banana varieties is depicted in Table 1. The total reduced glutathione content in banana pulp extracts ranged between 4.48 - 10.55 mg g⁻¹

fresh tissues. Rasthali banana exhibit highest content of total reduced glutathione among other varieties. On the other hand Red banana showed least activity.

Table 1
Total reduced glutathione content in pulp extracts of nine varieties of banana.

Banana varieties	Total reduced glutathione
Kadali	6.53 ^b ± 0.345
Karpooravalli	9.26 ^f ± 0.172
Nendran	7.06 ^c ± 0.165
Monthan	8.42 ^e ± 0.225
Pachainadan	8.57 ^e ± 0.116
Poovan	9.56 ^f ± 0.23
Rasthali	10.55 ^g ± 0.346
Red	4.48 ^a ± 0.401
Robusta	7.74 ^d ± 0.23

Values represent mean ± SD of 3 replicates. Means followed by a common letter are not significantly different at the 5% Level by DMRT. Units: Total GSH – mg g⁻¹ fresh tissue

Estimation of vitamin A

Vitamin A or retinol is a polyisoprenoid compound containing a cyclohexanyl ring. It is stored mainly as retinol esters in liver. It plays

a role in trapping peroxy free radicals in tissues at low partial pressure of oxygen. It also involved in the regulation of lipid peroxidation in plasma.

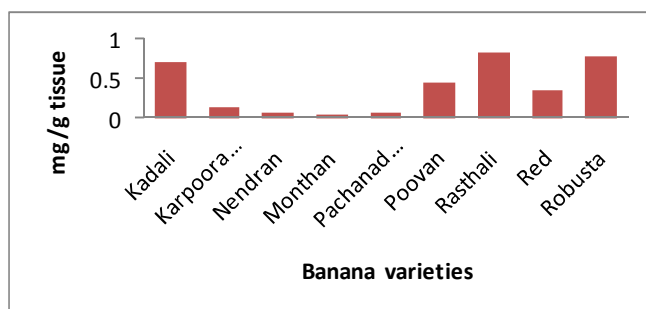
Table 2
vitamin A content in pulp extracts of nine varieties of banana.

Banana varieties	Vitamin A
Kadali	0.705 ^f ± 0.006
Karpooravalli	0.135 ^c ± 0.005
Nendran	0.069 ^b ± 0.006

Monthan	0.050 a 0.006
Pachainadan	0.075 b±0.008
Poovan	0.448e ±0.007
Rasthali	0.835 h 0.005
Red	0.348 d±0.009
Robusta	0.782 ^g ±0.003

Values represent mean \pm SD of 3 replicates. Means followed by a common letter are not significantly different at the 5% Level by DMRT. Units: Vitamin A - mg vitamin A acetate equivalent g⁻¹ fresh tissue.

Figure 2.
Vitamin A content in pulp extracts of nine varieties of banana.



As described in table 2. Vitamin A levels varied from 0.050 – 0.835 mg g⁻¹ fresh tissue. The highest vitamin A content recorded in Rasthali banana compared to other varieties and least amount recorded in Monthan banana.

Estimation of Vitamin C

Vitamin C or ascorbic acid one of the most important vitamin that is supplied by fruits

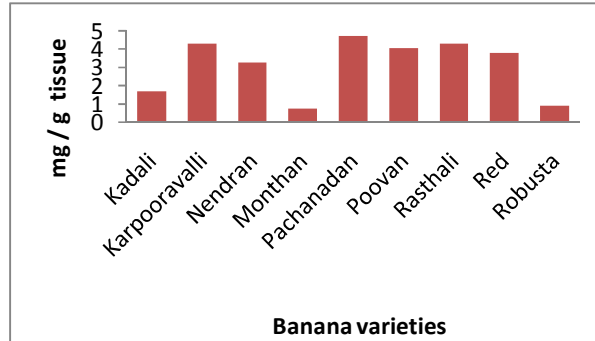
and vegetables. It is a major soluble antioxidant within the body [9]. It lowers the blood pressure and cholesterol levels.

Table 3
Vitamin C contents in pulp extracts of nine varieties of banana.

Banana varieties	Vitamin C
Kadali	3.27 d \pm 0.230
Karpooravalli	4.03 f \pm 0.016
Nendran	4.29 g \pm 0.068
Monthan	1.67 c \pm 0.052
Pachainadan	3.79 e \pm 0.137
Poovan	4.69 ^h \pm 0.085
Rasthali	0.88 b \pm 0.013
Red	4.26 g \pm 0.070
Robusta	0.71 a \pm 0.007

Values represent mean \pm SD of 3 replicates. Means followed by a common letter are not significantly different at the 5% level by DMRT. Units: Ascorbic acid – mg g⁻¹ fresh tissue.

Figure3
Vitamin C content in pulp extracts of nine varieties of banana.



The vitamin C content in pulp extracts of nine varieties of banana is furnished in Table 3. The vitamin C content from pulp extract of nine varieties of banana varied from 0.71-4.69 mg g⁻¹ fresh tissue. The highest vitamin C content was present in Poovan banana and on the other hand least content was found in Robusta.

Estimation of Vitamin E

Vitamin E is a fat-soluble vitamin and one of a number of nutrients called antioxidants. Other well-known antioxidants are vitamin C and

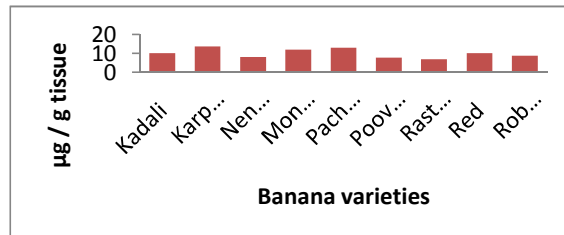
beta-carotene. Antioxidants are nutrients that block some of the damage caused by toxic by-products released when the body transforms food into energy or fights off infection.

Table 4
Vitamin E contents in pulp extracts of nine varieties of banana.

Banana varieties	Vitamin E
Kadali	10.11e 0.110
Karpooravalli	13.73h 0.172
Nendran	8.09 c ±0.160
Monthan	11.84f ±0.142
Pachainadan	12.99 ^g ±0.344
Poovan	7.83 ^b ±0.170
Rasthali	6.84 a ±0.104
Red	10.18e 0.165
Robusta	8.79 d±0.152

Values represent mean ± SD of 3 replicates. Means followed by a common letter are not significantly different at the 5% level by DMRT. Units: Vitamin E - µg α-tocopherol equivalents g⁻¹ fresh tissue.

Figure 4
Vitamin E content in pulp extracts of nine varieties of banana.



The Vitamin E content in pulp extracts of nine varieties of banana ranged from 6.84-13.73 µg g⁻¹ fresh tissue was shown in table 4. The highest vitamin E content was recorded in Karpooravalli banana and least activity was found in Rasthali banana.

CONCLUSION

The human body has been naturally blessed with a number of disease combating compounds that are sensibly programmed to act instantaneously. It is during the deficit of these substances that our body becomes afflicted with various ailments that may subsequently turn chronic. To prevent this, it is often recommended that people should intake natural supplements of these substances and antioxidants take the priority lead considering its valuable functions in the body. The present work has been undertaken to evaluate compare the non enzymatic content of nine varieties of banana viz. Kadali, Karpooravalli, Monthan, Nendran, Pachainadan, Poovan, Rasthali, Red and Robusta. The pulp extracts were assayed for

different enzymatic antioxidant compounds like vitamin A, vitamin C, vitamin E and total reduced glutathione, etc. The results obtained were subjected to two-way ANOVA and the varieties were ranked according to their enzymatic antioxidant content. All the nine banana varieties showed significant antioxidant enzyme content with Rasthali, Robusta, Poovan, karpooravalli topping the result.

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