

**β -CAROTENE, IRON AND ZINC CONTENT IN PAPUA NEW GUINEA AND
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ABSTRACT

Nutritional disorders due to inadequate intake of vitamin A, iron and zinc in the East African region are unusually high. Interventions to alleviate these deficiencies rely on supplementation and food fortification programs, which are not sustainable and do not reach all the affected. Sustainable solutions to malnutrition can be developed through linking agriculture, nutrition, and health. Promotion of consumption of bananas with enhanced micronutrient content as well as enriching bananas with micronutrients through breeding could go a long way towards preventing micronutrient deficiencies in the region, since bananas are a widely consumed staple. As a starting point in promoting consumption of bananas with enhanced micronutrients and breeding for higher micronutrient content in bananas, banana accessions both local and exotic hybrid Papua New Guinea (PNG) were screened. Pulp color intensity was measured, β -carotene was measured by high-performance liquid chromatography and iron and zinc were determined by atomic absorption spectrophotometry. β -carotene concentrations ranged from 92.3 $\mu\text{g}/100\text{g}$ to 2594.0 $\mu\text{g}/100\text{g}$, while iron and zinc concentrations ranged from 0.063 mg/100g to 0.608 mg/100g and 0.00003 mg/100g to 0.598 mg/100g, respectively. Banana pulp color intensity had a significant ($P<.0001$) positive correlation ($R=0.491$) with β -carotene concentration. Among PNG bananas, a significant positive ($P<0.01$) correlation ($R=0.633$), between β -carotene and pulp color also existed, while among the East African Highland cooking bananas, though positive the correlation ($R=0.066$) was not significant at $P=0.05$. Not only will accessions identified with relatively high concentrations of the three micronutrients be promoted for consumption, but will also be used as parents for population development through biofortification. It is expected, that through recombination and recurrent selection, micronutrient contents will be enhanced to concentrations that are significant for human nutrition. These results further highlight the importance of organoleptic characters like pulp color for choosing β -carotene dense bananas to combat health disorders caused by lack of inadequate intake of foods rich in vitamin A.

Key words: β -carotene, Deficiency, Bananas, Recurrent selection

INTRODUCTION

Banana (*Musa* spp.) is a major staple food crop providing about 25% of the carbohydrate requirements for millions of people in eastern Africa including Uganda, Rwanda, Burundi, eastern Democratic Republic of Congo, Tanzania and western Kenya [1]. It also serves as a source of income for millions of inhabitants of the region. Data indicate that the annual production of bananas within the region is estimated at about 20 million tonnes, with Uganda alone producing more than half of the region's production [2]. Also, data in the region indicate that Uganda has the highest *per capita* consumption of 500 g/per/day, followed by Rwanda, Burundi, Kenya, Democratic Republic of Congo and Tanzania [3]. Over 200 different banana varieties including those traditionally classified as juice, dessert, roasting and cooking are found in the region [2, 4, 5]. The dominant varieties are the East African Highland cooking types (AAA) that are used in the preparation of 'matooke', a popular local dish in Uganda [4].

The 5th report of World Nutrition Situation reveals that health disorders related to vitamin A deficiency (VAD) and iron deficiency anaemia (IDA) are documented in the east and central African region [6]. Vitamin A deficiency was defined as percentage with any VAD serum retinol below 0.7 μ mol/L, while anaemia was defined by haemoglobin level below 11.0 g/dl. Iron deficiency anaemia (IDA) prevalence has affected more than 50% of women in the past ten years while for children under five years it has risen from 35% in 1997 to more than 60% in 2000 [6, 7]. Children and women in rural areas and from lower income quantiles are reported to suffer more from VAD and IDA than others [6]. Data also indicate that VAD incidence among children under five is highest in Kenya and Uganda (70% and 66%, respectively) and least in Tanzania (37%) [8]. In Uganda, the banana-growing regions of central and western Uganda have the highest prevalence of VAD among women at 57% and 55%, respectively and the eastern region is not far behind with a prevalence rate of 52% [9]. Furthermore, findings in banana growing regions indicate that levels of zinc are low in banana-based weaning foods [10] and zinc intake in the general population is sub-optimal [11]. Several studies [10-14] suggest improving zinc nutrition should be made a priority, especially in regions where zinc is predominately lacking in their staple plant-based diets.

Although the majority of banana varieties are relatively rich in carbohydrate, fiber, vitamins C and B₆ [15, 16], they have low amounts of iron, iodine, zinc and vitamin A [17, 18]. The majority of children that are weaned primarily on cooking bananas in Uganda are exposed to diseases associated with iron, zinc, vitamin A and iodine deficiencies [10]. Zinc and vitamin A are important for the immune system, and iron and iodine are important for brain development *in utero* [19]. By promoting consumption of bananas with enriched micronutrients, severe deficiencies can be reduced in developing countries where diets are largely banana-based. Furthermore, increasing the nutritional value of bananas through breeding will enhance the health and well-being of the people [20]. However, variation of micronutrients in bananas grown in East Africa is not well documented and thus remains unknown.

The aim of this study was to investigate the variation of β -carotene, iron and zinc contents of samples of bananas grown in Uganda under similar conditions. Bananas sampled included: local East African Highland cooking varieties and the exotic bananas from Papua New Guinea (PNG). Papua New Guinea bananas were chosen because of their distinctively orange pulp, an indication of higher β -carotene content, as opposed to the yellow pulp of the East African Highland bananas.

MATERIALS AND METHODS

Sample identification, preparation and analysis

The 17 banana accessions used in the analysis are listed in Table 1. The white pulped hybrid 'TMBx5610' was sampled for comparison purposes. The hybrid 'TMBx5610' is from the cross of the East African Highland cooking banana 'Kabucuragye' and the hybrid '7197-2'. The accessions represent a range of different genotypes that were selected from the field on the basis of their pulp color. Three fingers were randomly picked from the harvested bunch and analyzed for β -carotene, iron and zinc at the Uganda Government Analytical Laboratory (UGAL) in Kampala. The protocol for β -carotene analysis in sweet potatoes [21] was adopted and modified according to observations described by Fraser *et al.* [22] to analyze the β -carotene content in bananas by using high performance liquid chromatography (HPLC). An atomic absorption spectrophotometer (AAS) was used to determine zinc and iron content according to procedures outlined by Okalebo *et al.* [23]. Banana samples were sliced lengthwise, to fit the sensor of the color meter (Color Tech. PCM_{TM} Pittsford, New York) firmly and flatly during the measurement of color intensity. As a result of different samples having different pulp coloration, the color meter was calibrated to measure the yellowness index, computed using the ASTM D-1925 equation. This was to avoid bias and mix up of sample color reflectances. Consequently, the displayed measurement was a specific calculation made using sample yellowness reflectance. The value of pulp intensity was displayed as Yellowness (D-1925). Each analysis was repeated three times. All data obtained were subjected to analysis of variance (ANOVA) and where significant differences were observed, means were separated using Fishers Protected Least Significant Difference (LSD) test at 5% probability level. Correlation analysis and level of significance ($P=0.05$) were done to determine the types of relationships between the β -carotene content with the pulp color intensity, iron and zinc. Data for β -carotene and color intensity were transformed to Log (10), to bring normality within the data set. Furthermore, correlations were done for the two categories of bananas of Papua New Guinea and the East African Highland cooking types.

RESULTS

β -carotene content and pulp color intensity

Preliminary results indicated that 8 of the 17 banana accessions had β -carotene levels above 1,000 $\mu\text{g}/100\text{g}$ (Table 1). The 8 accessions were exotic bananas from PNG that were obtained from the *Musa* germplasm collection at the International Banana

Transit Center in Belgium and grown in Uganda. Among the PNG accessions, the levels of β -carotene ranged from 204.9 $\mu\text{g}/100\text{g}$ in 'Pongani' to 2594.0 $\mu\text{g}/100\text{g}$ in 'Dimaemamosi'. On the contrary, the level of β -carotene in the East African Highland cooking bananas ranged from 99.8 $\mu\text{g}/100\text{g}$ in 'Kikundi' to 513.7 $\mu\text{g}/100\text{g}$ in 'Nakitembe'. The hybrid cultivar 'TMBx5610' had β -carotene content falling in the range registered by the East African Highland cooking types.

Pulp color intensity was measured (Table 1) to ascertain if there is any correlation between pulp color and β -carotene levels. Overall, results revealed a significant ($P < 0.0001$) positive correlation ($R = 0.49$) between pulp color intensity of banana pulp and β -carotene concentration (Figure 1). It was also found that among PNG bananas, a significant positive ($P < 0.01$) correlation ($R = 0.63$) between β -carotene and pulp color levels existed. On the other hand, among the East African Highland cooking bananas, although the correlation was positive ($R = 0.07$) it was not significant at $P = 0.05$ (Fig 2).

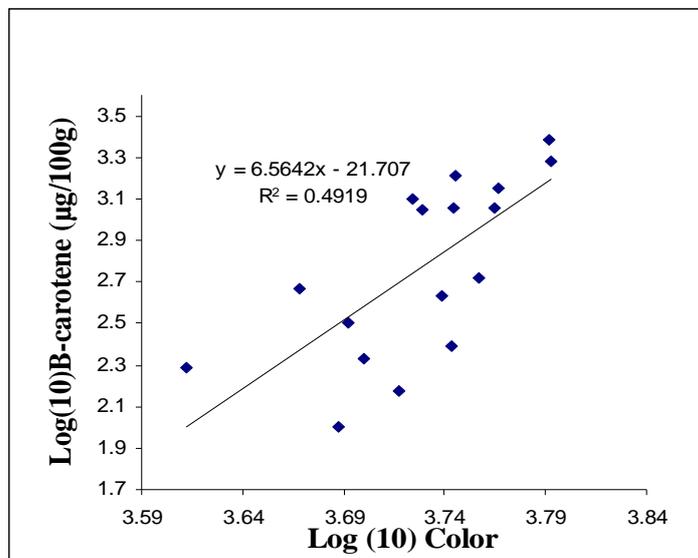


Figure 1: Relationship between pulp color intensity and β -carotene in 17 banana accessions

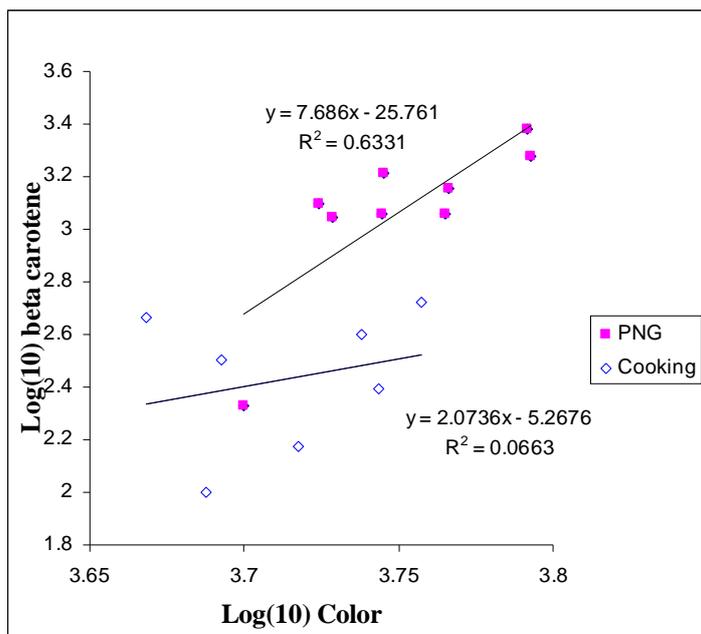


Figure 2: Relationship between β -carotene with pulp color of Papua New Guinea and East African Highland cooking banana accessions

Iron and zinc variation

The average iron and zinc contents in the pulp of the 17 banana accessions are illustrated in Table 1. The highest iron content (0.608 mg/100gms) was found in the East African Highland cooking banana of ‘Nakhaki’ and the lowest in ‘Kikundi’ (0.063 mg/100g). The highest zinc content (0.598 mg/100gms) was found in ‘Kokopo 1’ and the lowest in ‘Enzirabahima’ (0.00003 mg/100mg).

Correlation between β -carotene with iron and zinc

The correlation analysis revealed positive correlations between β -carotene with iron (R=0.098) and zinc (R=0.154) contents among the 17 banana accessions (Figures 3 and 4). However, the correlations were not significant at P=005. Also positive correlations were realized between β -carotene and iron or zinc content among the PNG and the East African Highland cooking bananas (Figures 5 and 6).

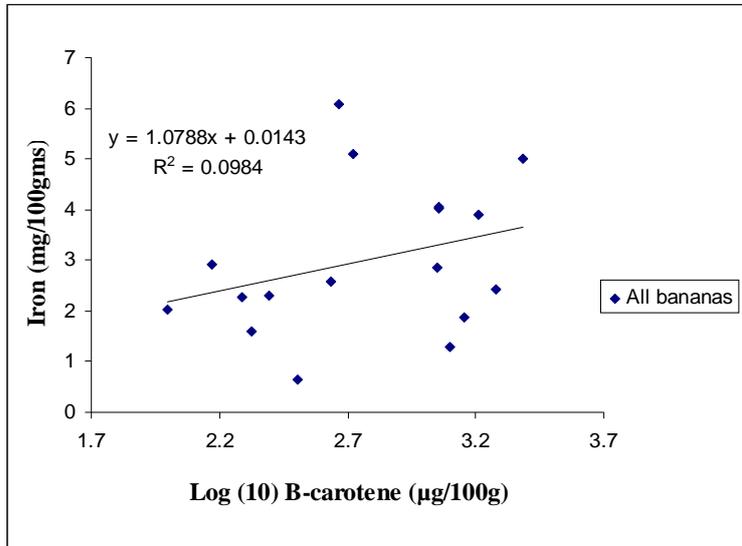


Figure 3: Relationship between iron and β -carotene in 17 banana accessions

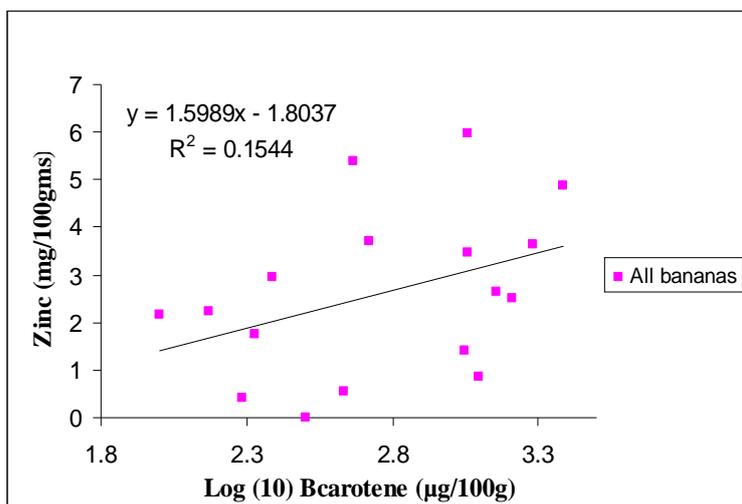


Figure 4: Relationship between zinc and β -carotene in 17 banana accessions

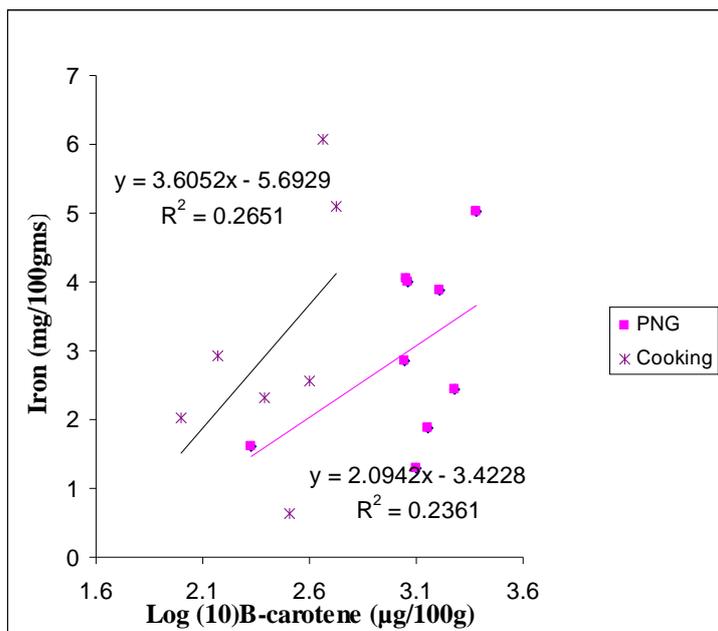


Figure 5: Relationship between β -carotene and iron in Papua New Guinea and East African Highland cooking banana accessions

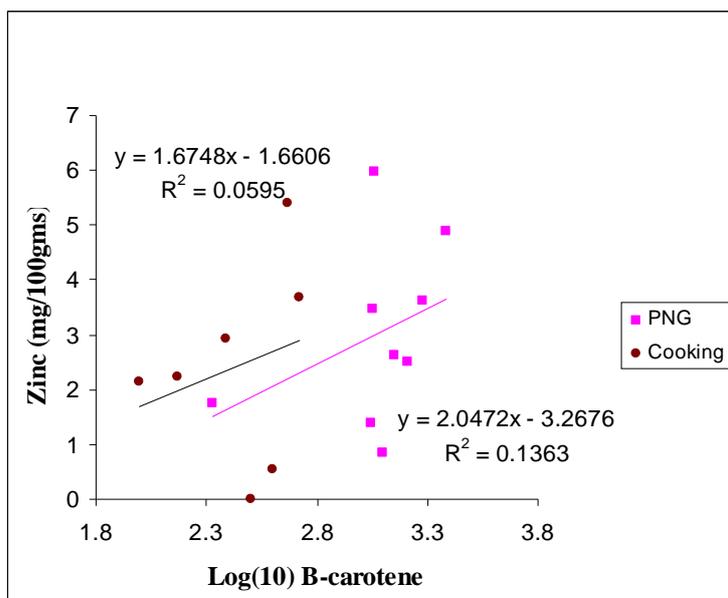


Figure 6: Relationship between β -carotene and zinc in Papua New Guinea and East African Highland cooking banana accessions

DISCUSSION

β -carotene content and pulp color intensity

Orange pulped Papua New Guinea accessions had a significantly higher β -carotene content than the yellow pulped East African Highland cooking banana accessions (Table 1). For instance β -carotene content in the PNG accessions was approximately 6 fold that of East African Highland cooking banana accessions. This compares well with the 5 fold β -carotene content between orange and yellow pulped Micronesian bananas reported by Englberger *et al.* [24, 25]. This implies that content of β -carotene is higher in orange pulped bananas than in yellow and creamy pulped bananas. Associating banana pulp color with β -carotene or vitamin A would help the vulnerable communities in selecting bananas with sufficient vitamin A content, thus reaping from the numerous nutritional and health benefits contributed by vitamin A [26-28]. However, further studies, investigating more color parameters and more pro-vitamin A carotenoids are essential, to explain in detail the nature of the relationship between vitamin A and pulp coloration in banana.

Conversely, using the World Health Organization [29] estimations for the amounts that may be commonly consumed, it is possible to look at the potential impact of the bananas in this study on meeting vitamin A and mineral requirements for children and women. A child of 2-5 years old consuming 250 g of food daily would obtain his total daily retinol requirement of 200 μ g RE [29] from one of the following raw cultivars: 'Dimaemamosi', 'Gunih', 'Galeo', 'Kokopo 1', 'Wambo', 'Pisang Mas', 'Yalim', 'M.acuminata ssp. Malacensis' and 'Nakitembe'. Also, a lactating mother consuming 500g daily would also obtain her total daily retinol requirements of 450 μ g RE by consuming 500g of 'Dimaemamosi', 'Gunih', 'Galeo', 'Kokopo 1', 'Wambo', 'Pisang Mas', 'Yalim' and 'M.acuminata ssp. Malacensis'. The PNG bananas, though not consumed in Uganda could be used as a source of beta carotene in banana improvement programs.

Iron and zinc variation

The pulp mineral content among the PNG bananas is comparable to the one reported in previous studies. Siong [30] reported an iron content of 0.6 mg/100g among PNG bananas of 'Pisang Mas', which is comparable to 0.4 mg/100g registered in 'Pisang Mas' in this study. Also, results among Micronesian bananas reveal that the banana cultivar 'Uht karat' had 0.2mg/100g of iron and 0.3 mg/100g zinc content [25]. On the other hand, the mineral analysis findings show that the East African Highland cooking and PNG bananas cannot meet iron and zinc average daily mineral requirements for a 2-5 year- old child and a lactating mother. A 2-5 year old- child consuming 250 g of food daily would obtain his/her daily iron requirement (5.05 mg/day) [29] from cooking cultivars of 'Nakhaki' and 'Nakitembe' and his/her total zinc requirements (4.45 mg/day) [29] from cultivars including: 'Kokopo 1', 'Nakhaki', and 'Dimaemamosi'. Results further reveal that if a similar comparison for a lactating mother consuming about 500 g of food per day is done, she could not obtain her total daily iron requirement (12.5 mg/day) [29] from the cultivars analyzed in this study. However, a lactating mother would obtain her total daily zinc

requirements (5.133 mg/day) [29] from two cultivars including 'Kokopo 1' and Nakhaki'. The low mineral content in the East African Highland cooking bananas may explain the high prevalence levels of the mineral deficiencies in the banana growing regions of East Africa. However, studies on bioavailability of these minerals have not yet been carried out. Wastney and others [31] indicated that high amounts of inositol hexaphosphate (phytates) in plant-based foods have a strong potential of binding divalent cations and their depressive effect on mineral absorption has been demonstrated in humans [32]. Thus, further studies on the bioavailability of these minerals to confirm the contribution of banana-based foods to meeting iron and zinc requirements are needed.

Correlation between β -carotene and iron and zinc

The positive correlations between β -carotene with iron and zinc in this study may demonstrate co-existence of β -carotene with iron and zinc in bananas. This correlation may suggest interactions at the physiological levels. This may be attributable to co-segregation of genetic factors controlling the bio-synthesis of β -carotene, iron and zinc. Previous studies by Monasterio and Graham [33], and Graham and Rosser [34], revealed significant ($P=0.05$) positive correlations, between β -carotene with iron and zinc in wheat and cassava. However, further studies in bananas, detailing characterization, markers and genes controlling the synthesis of the micronutrients are needed. These would, therefore, confirm whether genetically selecting banana accessions for increased β -carotene content may also result in increases in iron or zinc in the pulp.

CONCLUSION AND RECOMMENDATIONS

These findings are of particular importance in the banana growing regions of East and Central Africa, where bananas are easily grown, highly acceptable and where cultivars with high levels of β -carotene, iron and zinc could contribute meaningfully to alleviating the high prevalence of nutritional disorders related to vitamin A, iron and zinc. For a start, an initiative of promoting consumption of bananas with nutrients that can meet the daily vitamin A and mineral requirements is recommended. Furthermore, bioavailability of the three nutrients needs to be investigated to confirm contribution of these cultivars to vitamin A, iron and zinc status.

Papua New Guinea bananas represent a basis for recombination to increase β -carotene and to a certain extent, iron and zinc content in cooking bananas to levels which are higher than the current ones. Recombination technology to increase β -carotene, iron or zinc content in other crops, such as cassava [35] and orange fleshed sweet potatoes [33], has been successful. Information on banana biofortification is still limiting its applications. Through cycles of recurrent selection, varieties with superior concentrations of the three nutrients will be bred. Identification of genes through use of markers, which control synthesis of the three micronutrients in bananas, needs to be carried out.

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Table 1: Accessions, ploidy, origin of bananas, pulp color, average and range of β - carotene, iron and zinc content, retinol equivalent, of 17 bananas used in this study

Local name	Ploidy	Origin	Color of pulp	color Intensity	Average & Range of β -Carotene content ($\mu\text{g}/100\text{g}$)	RE ^a ($\mu\text{g}/100\text{g}$)	Average and Range of iron content (mg/100g)	Average and Range of zinc content (mg/100g)
1. Dimaemamosi	AA	PNG	Orange	5724	2416.7 (2134.5-2594.0)	402.8	0.502 (0.32-0.731)	0.489 (0.371-0.623)
2. Galeo	AA	PNG	Yellowish-Orange	5086	1254.9 (1154.1-1312.6)	209.2	0.129 (0.122-0.135)	0.856 (0.079-0.091)
3. Gunih	AA	PNG	Orange	5706	1460.2 (1390.2-1581.3)	243.4	0.189 (0.179-0.203)	0.263 (0.261-0.264)
4. Kokopo 1	AA	PNG	Orange	5886	1141.8 (955.1-1271.5)	190.3	0.401 (0.395-0.410)	0.598 (0.525-0.712)
5. M.acuminata ssp. malacensis	AA	PNG	Yellowish-Orange	5183	1114.2 (998.7-1209.3)	185.7	0.286 (0.256-0.312)	0.139 (0.121-0.159)
6. Pisang Mas	AA	PNG	Orange	5555	1372.1 (1291.4-1512.0)	228.7	0.404 (0.389-0.494)	0.348 (0.321-0.373)
7. Pongani	AA	PNG	Light Yellow	5012	213.0 (204.9-256.8)	35.5	0.160 (0.135-0.184)	0.176 (1.75-0.181)
8. TMB5610	AA	Hybrid-Uganda	White	4123	193.3 (182.3-201.5)	32.2	0.228 (0.212-0.237)	0.04 (0.025-0.062)
9. Wambo	AA	PNG	Orange	5980	1904.4 (1677.7-2102.7)	317.4	0.244 (0.241-0.253)	0.364 (0.263-0.546)
10. Yalim	AA	PNG	Orange	5561	1560.6 (1489.6-1664.5)	260.1	0.389 (0.371-0.40)	0.252 (0.237-0.275)
11. Enzirabahima	AAA	Uganda	Yellow-Orange	4929	314.5 (301.6-321.2)	52.4	0.102 (0.045-0.092)	0.0003 (0.00-0.001)
12. Kibuzi	AAA	Uganda	Yellow-Orange	5472	428.9 (416.7-443.7)	71.5	0.257 (0.212-0.289)	0.054 (0.038-0.074)
13. Kikundi	AAA	Uganda	Yellow-Orange	4853	99.8 (92.3-109.5)	16.6	0.063 (0.018-0.022)	0.215 (0.189-0.231)
14. Mpologoma	AAA	Uganda	Yellow-Orange	5198	146.4 (142.8-151.2)	24.4	0.292 (0.281-0.306)	0.222 (0.145-0.272)
15. Nakhaki	AAA	Uganda	Light Yellow-Orange	4657	448.8 (423.1-466.2)	74.8	0.608 (0.519-0.684)	0.539 (0.513-0.578)
16. Nakitembe	AAA	Uganda	Yellow-Orange	5720	513.7 (489.1-531.9)	85.6	0.509 (0.441-0.552)	0.369 (0.335-0.388)
17. Tereza	AAA	Uganda	Yellow-Orange	5574	245.7 (232.2-253.5)	41.0	0.231 (0.219-0.237)	0.294 (0.225-0.386)
LSD				133.1	169.7	-	0.9845	1.0427

^aRE: Retinol equivalents (conversion factor 6:1 from β - carotene equivalents to RE)

Source:

(29)

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