

## **Retention of Provitamin A Carotenoids during Post-harvest Ripening and Processing of Three Popular *Musa* Cultivars in South-Western Uganda**

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### **Abstract**

Banana and plantain (*Musa* spp.) form an important part of the diet of communities in Uganda. Despite preliminary indications that some cultivars could be good sources of provitamin A carotenoids (pVACs), vitamin A deficiency remains a health problem in banana-dependent regions of Uganda. The most popular *Musa* cultivars in southwestern Uganda include two East African highland bananas (AAA-EA) 'Entaragaza' and 'Mbwazirume', and one plantain (AAB) 'Manjaya-Gonja'. The AAA-EAs are mostly eaten steamed, while the plantain is generally roasted. Retention levels of pVACs during fruit ripening and processing of these popular cultivars were determined by high performance liquid chromatography (HPLC). Highest levels were found for ripe 'Entaragaza' (7319 µg/100gdw), fully ripe 'Mbwazirume' (6493 µg/100gdw) and fully ripe 'Manjaya-Gonja' (13,377 µg/100gdw). Steamed 'Entaragaza' and 'Mbwazirume' retained more than 90% of total pVACs. Roasting, deep-frying and steaming of 'Manjaya-Gonja' resulted in substantial loss of pVACs, with the highest loss (58.5%) observed after deep-frying in fully refined vegetable oil. All-*trans* β- and all-*trans* α-carotene constituted over 87% of the total carotenoids. The plantain 'Manjaya-Gonja' had a significantly higher proportion of β-carotene, while the AAA-EAs had significantly higher proportions of α-carotene. Although no *cis* α-carotenes and only negligible levels of *cis* β-carotene were observed during ripening, after processing the proportion of *cis*-carotenoids increased to about 10%. Retinol Activity Equivalents (RAE) in the processed products ranged from 97.91 to 138.16 µg/100g edible portion. Therefore, consumption of 100 g of the tested products theoretically meets 24-35% and 16-20% of the vitamin A RDAs of a preschooler and a woman of reproductive age. Although steaming retains the highest levels pVACs, the tested *Musa* cultivars can make substantial contribution to vitamin A requirements of vulnerable groups whether roasted, boiled or fried.

### **INTRODUCTION**

Banana and plantain (*Musa* spp.) form a major staple food and source of income and employment for millions of people in sub-Saharan Africa. The most predominant types in East Africa, and especially Uganda, are the East African highland bananas (AAA-EA) and the plantains (AAB). The majority are consumed locally in various forms, such as cooked green, cooked ripe, cooked in the peel, steamed, made into juice, ripened for dessert, roasted, chipped and fried or dried and floured to make a host of

confectionaries (Karamura et al., 1996; Karamura, 1998; Lescot, 1999). In other words, there is an affordable banana dish for virtually every income category of consumers in the region (HarvestPlus, 2007), and consumption levels have been reported to be as high as 304 kg/person/year (FAO, 2001).

Recent analysis of the micronutrient content of 171 *Musa* genotypes suggests that some cultivars contain relatively high amounts of provitamin A carotenoids (pVACs) (Davey et al., 2009). An analysis of common cultivars from Eastern Democratic Republic of Congo (DRC) has also indicated that some can contribute substantially to the recommended dietary allowances (RDA) of vitamin A of a child below 5 years and a woman of reproductive age (Ekesa et al., 2011). Despite this, 20% of children aged 6-59 months, and 19% of women of child-bearing age are vitamin A deficient in Uganda (SCN, 2010), with deficiency levels being more predominant among rural banana-dependent populations, especially where children are weaned primarily on AAA-EA (Kikafunda et al., 1996).

Effective, culturally appropriate, food-based strategies are essential for sustainable solutions to alleviating vitamin A deficiency (VAD) (Ayewole-Olusola and Asagbra, 2003). These strategies empower individuals and households, leading to family food production, wise food selection and preparation methods, simultaneous provision of multiple nutrients and an enhancement of cultural pride and identity (Englberger et al., 2003a).

In this study, we have analyzed the levels of pVACs in fruit of two of the most popular AAA-EA cultivars and one popular plantain in Uganda. The aim was to characterize the changes that occur in pVACs content as the fruit ripens and the degree of pVACs retention following fruit processing and cooking. This information can be used to determine the potential contribution of *Musa* fruit and foods to the RDA for vitamin A and to guide consumer consumption patterns to maximize vitamin A intake for improved health in these regions.

## **MATERIALS AND METHODS**

### **Sample Collection**

The two most popular AAA-EA cultivars in south-western Uganda are 'Entaragaza' and 'Mbazirume', while the most popular plantain is 'Manjaya', also called 'Gonja' (Miroir, 2010). Mature, disease-free plants of these cultivars were identified in farmer fields by using botanical descriptors and marked by qualified agronomists. When the fruits of the bunch were mature [deep green, full and rounded or ripening stage 1, according to Dadzie and Orchard (1997)], the bunch was harvested, and the two middle hands (2<sup>nd</sup> and 3<sup>rd</sup> hand), each containing 12-16 fingers, were sampled. The samples arrived at the laboratory in Kampala within 48 hours of harvest. The ripening stage was estimated based on the peel color as described by Stover and Simmonds (1987), as follows: 1 = green; 2 = green with trace of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = only green tips remaining; 6 = all yellow; 7 = yellow flecked with brown. Ripening stages used in this study were stages 1, 3, 5 and 7.

### **Sample Preparation**

**1. Samples for analysis during ripening.** The harvested fruits were left in a well-aerated room to ripen naturally. For each cultivar, sub-samples consisting of two fingers were randomly selected at ripening stages 1, 3, 5 and 7. Fruits were cleaned, peeled and

quartered, the quarters of each finger placed in a labeled zip-lock plastic bag, the air removed manually and the bags stored at -20°C until lyophilization (maximum 2 weeks).

**2. Samples for analysis after processing.** Fruits were processed (prepared) into the most common products/dishes, making use of ingredients obtained from local markets, as described in Table 1. The preparation methods (recipes) tested included steaming (in banana leaves and in polythene), roasting and deep-frying. The AAA-EA cultivars were processed at ripening stage 3, while the plantain was processed at ripening stage 5. Approximately 100 g fresh weight (gfw) of the products/dishes were transferred to labeled zip-lock plastic bags, the air removed manually and the bags stored in a freezer at -20°C until lyophilization and analysis.

All collected samples were then lyophilized for 72 hours, re-packaged in their labeled zip-lock plastic bags with air removed manually and stored in the dark for transport as hand luggage by air to the Laboratory of Fruit Breeding and Biotechnology, Department of Biosystems at the *Katholieke Universiteit Leuven* (Belgium), for pVACs analysis. Diagonally opposite quarters of each sample of the lyophilized raw fruit pulp and the whole lyophilized sample of each product/dish were homogenized to a fine powder by grinding in liquid nitrogen in a pestle and mortar and stored in sealed tubes in the dark at -20°C until analysis.

### **Extraction and Carotenoid Analysis**

**1. Buffers and solvents.** HPLC-grade tetrahydrofuran (THF) and *tert*-butyl methyl ether (t-BME) were obtained from Sigma Aldrich, (Bornem, Belgium) and HPLC-grade methanol (MeOH) from LabScan (Dublin, Ireland). Lutein, all-*trans*  $\beta$ -carotene (t-BC), all-*trans*  $\beta$ -8-apocarotenal (apocarotenal), butylated hydroxytoluene (BHT), triethylamine and insoluble polyvinylpolypyrrolidone (PVPP) were all obtained from Sigma Aldrich.

**2. Extraction method.** All extractions were carried out in triplicate according to procedures specifically developed for the analysis of *Musa* tissues. In brief, around 100 mg aliquots of powdered, lyophilized fruit pulp were homogenized for 30 s at maximum speed with 3-5 glass beads in a 'FastPrep' reciprocal shaker in 400  $\mu$ l of ice-cooled extraction buffer. The extraction buffer consisted of THF:MeOH, 1:1 (v/v), containing 0.25% BHT and 2% insoluble PVPP (Davey et al., 2006, 2009a). Following centrifugation (14,000 rpm for 20 min at 4°C), the pellet was re-extracted twice more with 400  $\mu$ l of THF:MeOH, 1:1 (v/v), containing 0.25% BHT without PVPP. After each extraction step, the supernatant was collected and combined.

**3. Carotenoid analysis.** The combined supernatants were directly analysed by RP-HPLC using a Waters Alliance, 2690 Separations System fitted with an autosampler, thermostated at 8°C, a pulse dampener and a 996 UV-Vis photodiode array detector (Waters, Massachusetts, USA). The entire system was controlled and the data were collected and integrated using the Millennium 4.0 software package. Extracts were resolved on a 150 x 4.6 mm, YMC C<sub>30</sub> 3- $\mu$ m particle size HPLC column (Achrom, Zulte, Belgium), using a 24-minute linear gradient of 2-50% t-BME in MeOH at 1.0 ml/min and regenerated with 95% t-BME in MeOH. All buffers contained 0.05% BHT and 0.05% triethylamine. Peaks were quantified at 450 nm using a freshly-prepared standard curve in extraction buffer as described by Davey et al. (2006) and identified on the basis of their characteristic absorption spectra. Retention times relative to known standards were available (Azevedo-Meleiro and Rodriguez-Amaya, 2004; Davey et al., 2006; Howe and Tanumihardjo, 2006).

## Assessment of Carotenoid Content and Impact on Daily Vitamin A Recommended Dietary Intakes (RDIs)

The relative vitamin-A activity of 13 *cis*- $\beta$ -carotene (c-BC) has been estimated to be 53% of that of t-BC (Schieber and Carle, 2005), while that of t-AC is only 50% of the activity of t-BC (Trumbo et al., 2003; Fraser and Bramley, 2004). The mean total pVAC values derived from the results of three independent HPLC analyses at each of the four ripening stages were converted into t-BC equivalents [t-BCE, presented in nmol/g dry weight (gdw)], using the formula 't-BCE = 0.5 t-AC + t-BC + 0.53 c-BC'. Various studies have indicated that the moisture content in plantain (French horn) and AAA-EA cultivars is approximately 65% and 70% respectively, when the fruit is unripe (Agunbiade et al., 2006; Amankwah et al., 2011). In addition, during ripening there is a decrease in moisture content of the peel and an increase in moisture content of the pulp (Dadzie and Orchard, 1997). The increase in *Musa* pulp moisture content from unripe (ripening stages 1-3) to ripe (ripening stages 4-6) and from ripe (4-6) to fully/overripe (> ripening stage 7) is approximately 4% and 3%, respectively (Adeyemi et al., 2009; Ahenkora et al., 1997). Therefore to obtain the edible portion BCE content, 70% (fruit processed while unripe) and 69% (processed ripe) moisture contents were considered for the AAA-EA and plantain cultivars, respectively. The BCE values were then converted into 'Retinal Activity Equivalents' (RAE) assuming that 1/12<sup>th</sup> of the total t-BCE ingested are taken up into the body (Yeum and Russell, 2002). The RAE were compared to the daily RDIs for vitamin A in population groups particularly vulnerable to VAD, which according to McLaren and Frigg (2001) include children between 1 and 5 years and women of reproductive age<sup>1</sup>. The RDAs for these groups are 400  $\mu$ g/day and 700  $\mu$ g/day respectively (FAO/WHO, 2002).

### Statistical Analysis

Differences in the mean contents of total and individual pVACs and BCE between cultivars at the different ripening stages were tested by analysis of variance, and differences between cultivars determined by Tukey's HSD multiple rank test.

## RESULTS AND DISCUSSION

### Changes in Total and Individual pVACs during Ripening

The principal carotenoids observed in both the raw fruit and the processed fruit were t-BC and t-AC, although small quantities of lutein were also present in most of the samples. Only minor amounts of the 13-*cis* isomers of t-AC and t-BC were detected. This same observation has previously been made by Davey et al. (2009), Englberger et al., (2003a, 2003b, 2006) and Ngoh-Newilah et al. (2008, 2009). The mean total pVACs was calculated as the sum of the concentrations of t-AC, t-BC, and *cis*-carotenoids (13-*cis* BC and 13-*cis*-AC). The total pVACs ranged from 627.8  $\mu$ g/100gdw in 'Entaragaza' at ripening stage 1 to 13,376.9  $\mu$ g/100gdw in 'Manjaya-Gonja' at ripening stage 7 (see Table 2). Davey et al. (2009), when analyzing fresh fruit from 171 *Musa* cultivars, found total pVACs values ranging from 0 (undetectable) to 211 nmol/gdw (11,337  $\mu$ g/100gdw).

As the fruit ripened, the levels of both total and individual carotenoids changed, but the trends were different for the different cultivars (Table 1). In 'Manjaya-Gonja', there was a statistically significant ( $p=0.05$ ) decrease in total pVACs as the fruit began to

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<sup>1</sup> "Woman of reproductive age" is referring to a non-pregnant, non-lactating woman aged 15-45 years.

ripen (ripening stage 1-3), followed by a slight increase in total pVACs as the fruit progressed in ripening; the highest level was observed when the fruit was fully ripe; however, these later changes were not statistically significant (Fig. 1). This differs from the results of Ngoh-Newilah et al. (2008), who found that carotenoid levels in the plantain 'Batard' from Cameroon decreased as the fruit progressed in ripening. The levels of total pVACs in 'Mbazirume' rose gradually and no decrease was observed, with highest levels observed when the fruit was over-ripe (Fig. 2). In 'Entaragaza', there was a very significant rise in the level of total pVACs during the initial ripening stages, but as the fruit progressed in ripening, levels stabilized and then even decreased slightly, with the highest levels observed when the fruit was ripe (Fig. 3). The trend observed in 'Entaragaza' was similar to that observed in 'Popoulou-CMR' a cooking banana cultivar obtained from Cameroon (Ngoh-Newilah et al., 2008). In all three cultivars in this study, the pVACs values recorded when the fruit was fully ripe were higher than those observed immediately after harvest when the fruit was hard, firm and with green skin.

### **Changes in pVACs following Processing of *Musa* Fruit**

Reports have indicated that prolonged exposure to heat treatments such as deep-frying, boiling and a combination of several processing techniques can result in substantial losses of pVACs (Ruel and Bouis, 2004). In this study, good retention of pVACs was observed during steaming of the AAA-EA cultivars; in 'Entaragaza', pVAC retention was 90-96%, while in 'Mbwazirume', even an increase in PVACs (46-54%) was recorded. A possible explanation for this observed increase could be that steaming led to a loosening of the food matrix with more carotenoids being available as a result. In the plantain ('Manjaya-Gonja'), a different scenario was observed, as roasting, deep-frying and steaming all resulted in substantial loss in total pVACs, with the highest loss (58.5%) observed after deep-frying in fully refined vegetable oil 'Golden fry' (Figure 4). The carotenoids found in 'Manjaya-Gonja' thus appear to be less stable than those in 'Entaragaza' and 'Mbwazirume' and seem to be destroyed by heat more easily. In addition, the high loss during deep-frying concurs with reports that have indicated that deep-frying, especially for prolonged periods, results in substantial losses in pVACs (Rodriguez-Amaya, 1997).

Results also showed that the amounts of cis-carotenoids were higher in the processed banana samples as compared to the raw samples. No cis  $\alpha$ -carotenes and only negligible levels of cis  $\beta$ -carotene were observed in the three banana cultivars during ripening, but after processing the proportion of cis-carotenoids increased to about 10%. This same observation was made when carrots were heated; this indicates that the conversion of *trans* to *cis* isomers is stimulated by the heat during processing (Booth et al., 1992).

### ***Musa* pVACs Profile**

The carotenoids t-BC and t-AC constituted over 87% of total carotenoids in both the raw and the processed banana cultivars; this same observation was made by Davey et al. (2009) and Ekesa et al. (2011) (Figs. 5, 6). However, these findings are quite different from the profiles reported for other crops, such as maize and wheat, where the pVACs generally represent only 10-20% of the total tissue carotenoid content (Ortiz-Monasterio

et al., 2007) and where the major carotenoids are lutein and zeaxanthin (Leenhardt et al., 2006).

The vitamin A nutritional value of banana and plantain fruits depends not only on the concentration of pVACs but also on the relative proportion of the individual pVACs present (Trumbo et al., 2003; Fraser and Bramley, 2004). High t-BC contents are a preferred trait as t-BC is the pVAC that is most efficiently converted into retinol; t-AC has only 50% of the vitamin A activity of t-BC. Cis-isomers also possess different biological properties such as decreased provitamin A activity, and antioxidant capacity (Schieber and Carle, 2005).

In the plantain ('Majaya-Gonja'), the proportion of t-BC was significantly higher than that of t-AC in both the raw and the processed banana cultivars (Figs. 5, 6). In the two AAA-EA cultivars ('Entaragaza' and 'Mbwazirume'), the proportion of t-AC was significantly higher than that of t-BC. 'Manjaya-Gonja' therefore represents a preferred source of carotenoids as compared to the two AAA-EA cultivars for equivalent levels of total carotenoids.

### **Impact of Processed *Musa* Fruit on Vitamin A Requirements of Children and Women**

Following processing/cooking of the three *Musa* cultivars, the RAE values ranged from 97.91 µg/100g to 138.16 µg/100g. The contribution of the processed *Musa* cultivars to the vitamin A RDIs of a child (6-59 months) and a woman of reproductive age are summarized in Table 3. Results indicate that consumption of 100 g of steamed AAA-EA cultivars would meet more than 27% and 15% of the daily vitamin A requirements of a child below 5 years and a woman of reproductive age, respectively. Steamed, roasted or fried plantain would meet more than 24% and 14% of the daily vitamin A requirements of the respective target groups.

These findings confirm that there already exist *Musa* cultivars with sufficiently high pVACs contents to immediately have a noticeable impact on populations at risk of VAD at modest and realistic consumption levels. There is therefore a need to further evaluate the intra-household food distribution to further verify how much of the food prepared in the household is actually consumed by the specific vulnerable groups (preschool children and women of reproductive age). It is also important that awareness is created on best practices with regards to post-harvest practices (storage, processing and cooking) that optimize retention of vitamin A. In addition, additional bioaccessibility and bioavailability studies of the *Musa* products/dishes will help determine the actual contribution of the cultivars to the vitamin A requirements of the vulnerable groups.

This study only took into consideration the most popular plantain and two most popular AAA-EA cultivars from south-western Uganda, and only evaluated their potential contribution to vitamin A needs. There are over 60 *Musa* cultivars in Uganda (Karamura, 1998) and research has indicated that there is always nutrient-nutrient interaction, e.g. an increase in dietary fat content increases carotene absorption and protein deficiency decreases intestinal absorption of vitamin A. In addition, adequate intake of dietary fat and zinc is necessary for the absorption and utilization of vitamin A (Caballero, 1988). Therefore, there is a need to evaluate more cultivars and take into consideration the nutrition profile of both macronutrients and micronutrients, so as to establish whether the other nutrients present in the banana cultivars and dishes could either inhibit or enhance the availability and utilization of the vitamin A.

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## Tables

Table 1. Cooking procedures used in the processing of the three popular *Musa* cultivars from southwestern Uganda.

Processing method	Ingredients	Cooking procedure
Steaming 'Mbwarzirume', 'Entaragaza' and 'Manjaya-Gonja'	– 4 fingers per cultivar (480 g) – 500 ml water – banana leaves	Banana fingers were peeled with a knife and wrapped in cleaned young banana leaves. Strips and chunks of the banana leaves were placed at the base of the cooking pan as a foundation to minimize water getting into the wrapped bananas. The wrapped bananas were then placed on the foundation and water added, more leaves were added to cover and contents sealed with another cooking pan. The bananas were steamed over medium heat for 1 hour. The bananas were taken from the heat, unwrapped and while still hot were pressed together using banana leaves. The bananas were wrapped again in the same leaves and put back on the heat for about 20 minutes. The steamed, mashed bananas were then left to cool, unwrapped, about 100 g taken and placed in labeled zip-locked plastic bags with air removed manually and stored at -20°C. Comment: The three cultivars were cooked separately.
Roasting 'Manjaya-Gonja'	– 4 fingers (600 g)	A hot charcoal grill was prepared, and medium heat maintained. The ripe banana fingers were hand-peeled, placed on the grill and roasted under low heat till an even light brown skin formed. The fingers were left to cool, quartered and put in labeled zip-lock plastic bags with air removed manually and stored at -20°C.
Deep frying 'Manjaya-Gonja) in vegetable oil	– 4 fingers – local palm oil/cooking oil (Golden fry) – salt	The ripe plantain fingers were hand-peeled, cut into ¼ - ½ inch rounds. In a heavy saucepan, the vegetable oil was heated over medium heat and once it sizzled when a slice of plantain was added, several plantain slices were added and browned for about 2 min. They were transferred into a bowl to drain the excess oil and cool. They were put in labeled zip-lock plastic bags with air removed manually and stored at 20°C.

Table 2. Content and changes in individual and total provitamin A carotenoids in three *Musa* cultivars from Uganda.

Cultivar	Ripening stage	t-AC (nmol/ gdw)	t-BC (nmol/ gdw)	Lutein (nmol/ gdw)	c-BC (nmol/ gdw)	Total pVACs (nmol/ gdw)	T-BCs (nmol/ gdw)	Total pVACs (µg/ 100 gdw)
Entaragaza	Stage 1	7.65	4.04	0.29	0.00	11.69	8.18	628
	Stage 3	75.89	52.06	2.49	1.10	129.05	93.72	6,930
	Stage 5	78.56	57.49	1.92	0.25	136.30	100.10	7,319
	Stage 7	72.58	58.81	0.85	0.38	131.76	98.25	7,075
Mbwazirume	Stage 1	12.03	8.04	0.33	0.00	20.07	14.54	1,078
	Stage 3	49.34	39.55	2.14	0.00	88.90	66.22	4,774
	Stage 5	55.25	43.84	1.53	0.00	99.09	73.71	5,321
	Stage 7	68.57	51.69	2.02	0.65	120.91	89.13	6,493
Manjaya (Gonja)	Stage 1	80.12	110.43	0.00	0.13	190.68	153.81	10,239
	Stage 3	63.07	90.67	0.00	0.61	154.36	125.12	8,289
	Stage 5	91.31	122.36	0.38	0.32	213.99	171.90	11,491
	Stage 7	112.41	135.61	0.80	1.08	249.10	197.00	13,377

t-BC = all-*trans* β-carotene, t-AC = all-*trans* α-carotene, c-BC = all-*cis* β-carotene, T-BC=Total β-carotene, pVACs = provitamin A carotenoids, gdw = grams of dry weight.

Table 3. Contribution of processed *Musa* cultivars to the daily vitamin A recommended dietary intakes of a child (6-59 months) and a woman of reproductive age (15-45 years).

Cultivar	Product/dish	Total BCE (µg/100 gdw)	Total BCE µg/100 gep	RAE (µg/100 gep)	% RDI child <5 yrs	% RDI F 15-45 yrs
Enteragaza	Steamed in banana leaves	4,336.72	1,301.02	108.42	27.10	15.49
	Steamed in polyethylene	4,671.91	1,401.57	116.80	29.20	16.69
Mbwazirume	Steamed in banana leaves	5,292.83	1,587.85	132.32	33.08	18.90
	Steamed in polyethylene	4,992.97	1,497.89	124.82	31.21	17.83
Manjaya (Gonja)	Steamed in banana leaves	3,997.46	1,239.21	103.27	25.82	14.75
	Steamed in polyethylene	4,587.05	1,421.99	118.50	29.62	16.93
	Deep fried in vegetable oil	3,790.02	1,174.91	97.91	24.48	13.99
	Roasted	5,348.03	1,657.89	138.16	34.54	19.74

BCE = β-carotene equivalent, RAE = retinal activity equivalent, RDI = recommended dietary intake, F = female, gdw = grams of dry weight, gep = grams of edible portion.

## Figures

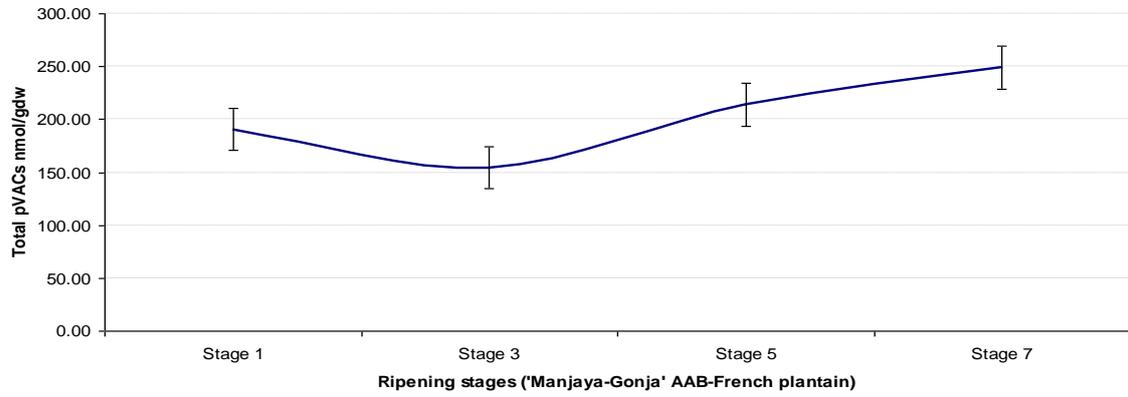


Fig. 1. Changes in total provitamin A carotenoids (pVACs) in banana cultivar 'Manjaya-Gonja' (AAB, plantain) during ripening.

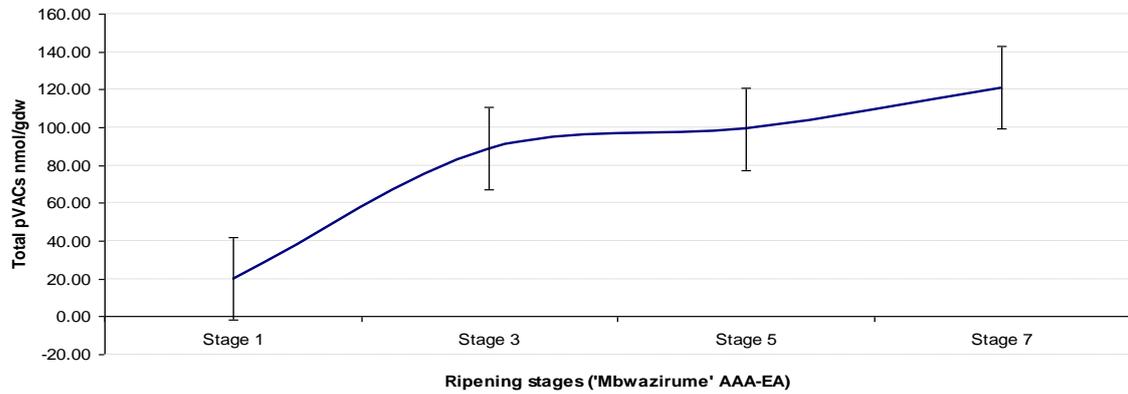


Fig. 2. Changes in total provitamin A carotenoids (pVACs) in banana cultivar 'Mbwazirume' (AAA-EA) during ripening.

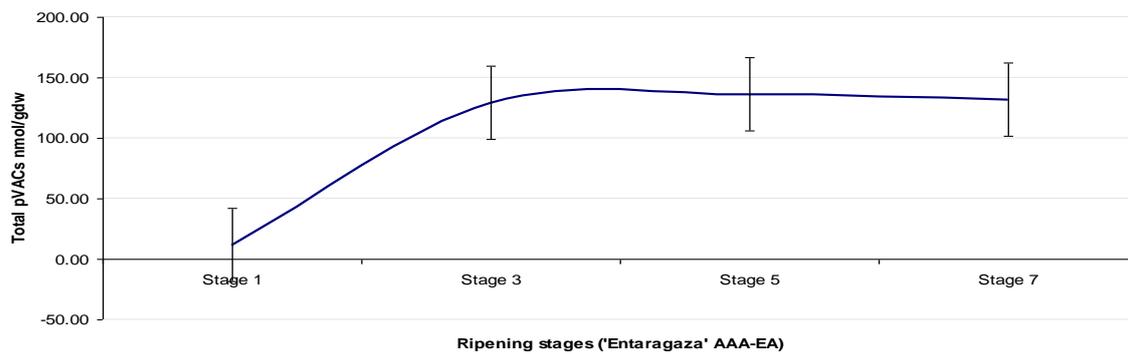


Fig. 3. Changes in total provitamin A carotenoids (pVACs) in banana cultivar 'Entaragaza' (AAA-EA) during ripening.

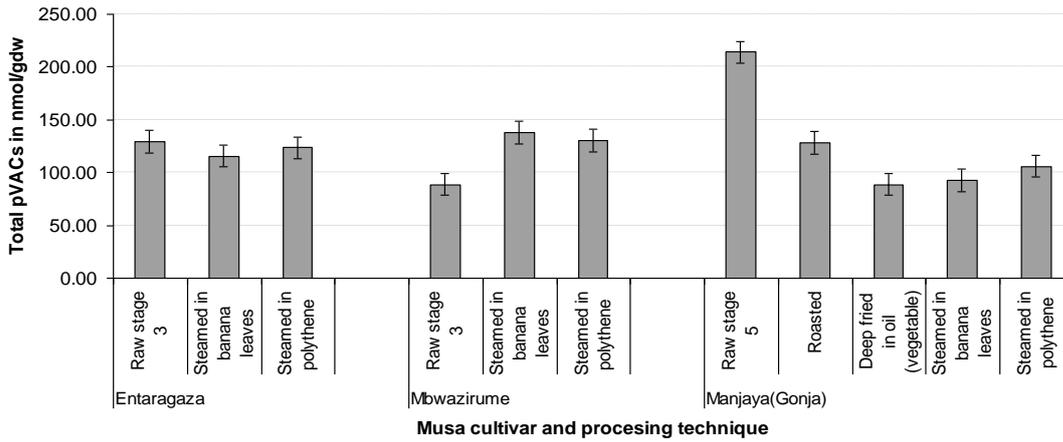


Fig. 4. Total provitamin A carotenoids (pVACs) level (nmol/gdw) in *Musa* cultivars following local processing.

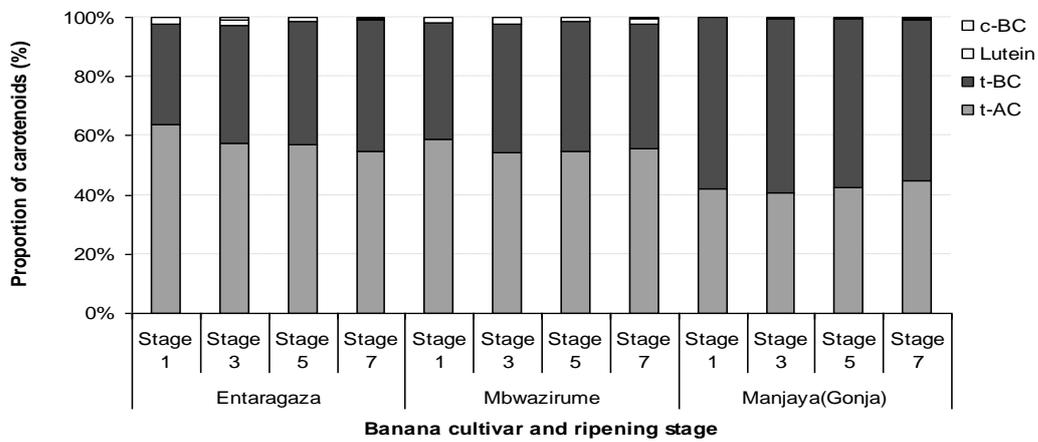


Fig. 5. Changes in proportion of different carotenoids during ripening of three popular *Musa* cultivars from southwestern Uganda.

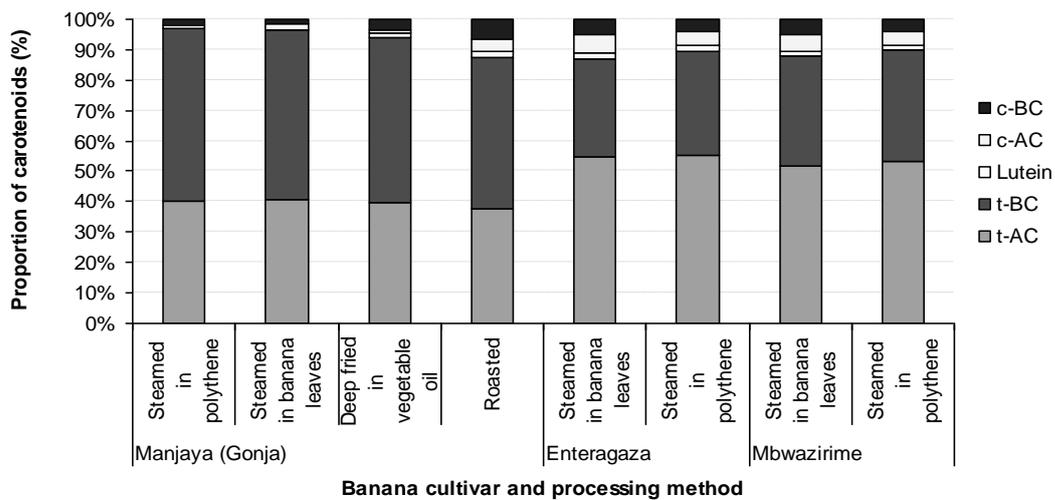


Fig. 6. Proportion of different carotenoids following processing of three popular *Musa* cultivars from southwestern Uganda.