Bioaccessibility of provitamin A carotenoids in bananas (*Musa* spp.) and derived dishes in African countries

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Abstract

Bananas and plantains (*Musa* spp.) constitute an important component of the diet in Africa. Substantial levels of provitamin A carotenoids (pVACs) in *Musa* fruit have been reported, but the bioaccessibility of these pVACs remains unknown. In this study**,** we used an *in vitro* digestion model to assess the bioaccessibility (i.e the transfer into micelles) of pVACs from boiled bananas and derived dishes using the Eastern Democratic Republic of Congo as a study context. In particular, the effect of different food ingredients added to boiled bananas on pVACs bioaccessibility was studied. The bioaccessibility of all-*trans* β-carotene ranged from 10 to 32% depending on the food recipes, and was modified particularly when pVACs-rich ingredients (palm oil/amaranth) were added. Efficiency of micellarization of all-*trans* β-carotene was similar to that of all-*trans* α-carotene and depended on the cultivar (Musilongo, plantain type, 16%; Vulambya, East African cooking type, 28%), while that of the 13-*cis* isomer was higher (21 to 33.5%). Tacking into account bioaccessibility, the estimated vitamin A activity was significantly different across the different *Musa*-based dishes tested. Results are discussed in terms of recommendations to help reduce vitamin A deficiency in *Musa*-dependent African communities.

Key words: East African highland banana, plantain, carotenoids, *in vitro* digestion model, plantain, vitamin A deficiency.

1. Introduction

Vitamin A deficiency (VAD) is a major public health problem in developing countries and it predominantly affects preschool children and women of reproductive age. In Eastern Democratic Republic of Congo (DR Congo), the World Health Organization (WHO) has reported a population VAD prevalence (as indicated by serum retinol levels of <20 µg/dl) of 61.1%. According to the classification by the International Vitamin A Consultative Group/Micronutrient Forum (also known as the Annecy Accords), a VAD prevalence of >30% indicates that VAD is a severe public nutrition problem (SCN, 2010). The traditional strategies of dealing with VAD include food fortification and supplementation and dietary diversity. However for rural agriculture-dependent populations, the primary source of vitamin A is derived from provitamin A carotenoids (pVACs) in plant foods (Ruel, 2001). There is thus a need to promote a higher dietary diversity, for instance through the production and consumption of dark-green leafy vegetables, yellow and red fruit and vegetables and red palm oil, which are all rich in pVACs. Effective, culturally appropriate, food-based strategies have been found essential for sustainable solutions to alleviating vitamin A deficiency (VAD) (Ayewole-Olusola & Asagbra, 2003). These strategies empower individuals and households thus leading to family food production, wise food selection and preparation methods. It also leads to simultaneous provision of multiple nutrients and an enhancement of cultural pride and identity (Englberger et al., 2003). Since diets of vulnerable groups especially preschool children are predominantly based on starchy staples with little or no fruits and vegetables or animal sources of preformed vitamin A, research has shown that breeding/fast tracking efforts targeting starchy staples with high levels of pVACs could have a great impact on VAD (Ayewole-Olusola & Asagbra, 2003).

In Eastern DR Congo, bananas (plantains and cooking bananas) constitute the second main starchy staple after cassava, with a consumption rate of around 137-174 kg/person/year (Dowiya, Rweyemamu & Maerere, 2009). Recently, research has highlighted the importance of dark-yellow/orange-fleshed *Musa* cultivars as a source of not only the well known calories but also pVACs (Davey, Keulemans & Swennen, 2006; Davey et al., 2007; Davey, Van den Bergh, Markham, Swennen & Keulemans, 2009; Englberger et al., 2003; Englberger et al., 2006). Generally higher levels of pVACs were found in plantains while other cooking bananas and commercial dessert types contain lower amounts (HarvestPlus, 2007). According to Davey, Kuelemans & Swennen, 2006; Davey, Stals, Ngoh-Newilah, Tomekpe, Lusty, et al., 2007; Davey, Van den Bergh, Markham, Swennen & Keulemans, 2009, some *Musa* cultivars can provide up to half of the total human daily vitamin A requirement in a single fruit.

The four most popular *Musa* cultivars in Eastern DR Congo include two plantains ('Musilongo' and 'Musheba', AAB genome) and two East African highland cultivars ('Nshikazi' and 'Vulambya', AAA-EA) (Ekesa, In press). An analysis of the pVACs contents in raw fruits of these four cultivars at different ripening stages has indicated that within normal consumption levels, these cultivars can contribute substantially to the daily vitamin A requirements of a child below five years and a woman of reproductive age[1]. The two cultivars from North Kivu ('Musilongo' and 'Vulambya') have significantly higher levels of pVACs than those (plantain 'Musheba' and AAA-EA 'Nshikazi') cultivated in South Kivu (Ekesa, In press). However little is known about the bioaccessibility of pVACs in these *Musa* fruits and/or derived dishes.

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[1] In this context a woman of reproductive age refers to a woman ages 15-45years who is neither pregnant nor lactating

Most *Musa* cultivars are processed into various forms, and studies from several other fruits and starchy staples have confirmed that heat processing influences carotenoids levels. In addition, after consumption, the release of carotenoids during digestion is determined by the extent to which the cell wall is degraded during processing (Tumuhimbise, Namutebi & Muyonga, 2009). The amount of food carotenoids released from the food matrix is commonly referred to as bioaccessibility and constitutes the maximum amount available for absorption by the enterocytes. Data on the bioaccessibility of pVACs is a critical step in determining the vitamin A activity of a particular food product. Previous studies have examined the effect of cooking on carotenoids bioaccessibility from fruits and vegetables, including numerous studies on cooked/processed orange-fleshed sweet potatoes (Ryan, O'Connell, O'Sullivan, Aherne & O'Brien, 2008; Failla, Thakkar & Kim, 2009; Bengtsson, Larsson-Alminger & Svanberg, 2009; Bengtsson, Brackmann, Enejder, Alminger & Svanberg, 2010). However, no research has looked at the effect of cooking on the carotenoids bioaccessibility of bananas and plantains. Estimation of *in vitro* carotenoids bioaccessibility, defined as the amount of carotenoids transferred from the food matrix to mixed micelles, allows a better understanding of how much of the provitamin A is ingested. The principle of an *in vitro* digestion model to screen bioaccessibility of carotenoids from food has been previously based on method of Garrett, Failla, Sarama, &Craft, 1999 and widely described by Failla, Tianyao & Thakkar, 2008. These authors reported the *in vitro* digestion process involving simulated oral, gastric and small intestinal digestion of test samples to access the efficiency of incorporation into micelles, an obligatory step for absorption of lipophilic compounds such as carotenoids. However, other authors optimized the procedure regarding pH and incubation times by taking data on lipid digestion and carotenoids processing *in vivo* into account (Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). The efficiency of carotenoids micellarization is affected by numerous factors, such as the matrix, processing/cooking method and fat, fiber or other additional components. Banana- and plantain-based dishes are often cooked together with additional ingredients such as vegetables, palm oil, green peas and beans which can influence carotenoids bioaccessibility.

The objectives of this study were twofold: to assess the bioaccessibility of pVACs in a boiled local plantain and cooking banana and to assess the bioaccessibility of pVACs in traditional dishes made from the two most popular *Musa* cultivars in Beni territory, North Kivu, Eastern DR Congo. To the best of your knowledge, provitamin A bioaccessibility from bananas have never been investigated for banana fruits, despite the great importance of this food for millions of poor people.

2. Materials and methods

2.1. Sample preparation and processing

The most popular East African highland banana (AAA-EA) and plantain cultivars in Beni territory Eastern DR Congo are 'Vulambya' and 'Musilongo', respectively (Ekesa, In press). Using botanical descriptors specific to these cultivars (Ekesa, In press), mature disease-free plants of these popular *Musa* cultivars were identified in farmers' fields and marked by qualified agronomists from Beni territory. Bunches were harvested at the mature stage when fruits were deep green, full and rounded (Dadzie & Orchard, 1997). Two middle hands (2nd and 3rd hand) each with between 12-16 fingers were subsequently sampled. The fresh fruit samples, as well as 'Musilongo' flour (processed in Beni territory using procedures described in table 1A-T5), were packed in a perforated carton box and transported under ambient temperature (20°C) from Beni, DR Congo to Kampala, Uganda. The samples arrived in Kampala within 24 hours after harvest. The most popular method of cooking banana and plantain among rural households in North Kivu is simple boiling; while additional ingredients added during the cooking process include fresh beans, amaranth leaves and occasionally local palm oil (Ekesa, In press). In this study, olive oil was also added as control oil containing only trace/no carotenoids. All these ingredients were obtained from a local market in Kampala and together with the fruit samples and flour, transported under ambient temperature within 48hours from DRC to Montpellier, France. The four common dishes from 'Vulambya' [i.e. boiled with fruit peel, boiled without fruit peel, boiled with fresh beans (with or without olive/palm oil) and boiled with fresh beans and amaranth leaves (with or without olive oil)] were prepared when the fruit was at ripening stage 3 (i.e. green fruit peel color with yellow spots highlights). The two most popular dishes from 'Musilongo' [boiled with fruit peel and boiled without fruit peel (with or without olive oil)] were prepared at ripening stage 5 (i.e. fruits are ripe). These are the ripening stages at which these *Musa* cultivars are normally processed by the community before consumption (Ekesa et al, 2011). All the dishes including 'Musilongo' porridge were prepared using local ingredients and following the procedures described by community members from North Kivu (Table 1). One replication of each dish was made. All dishes were subsequently stored at -20^{0}C under nitrogen awaiting analysis and *in vitro* digestion; storage did not exceed 3 days).

2.2. Chemicals

The extraction solvents were analytical grade and were purchased from Carlo-Erba (Val de Reuil, France), while analytic solvents were HPLC-grade methanol, acetonitrile and tetrahydrofuran (THF) also from Carlo-Erba (Val de Reuil, France), and methyl-tert-butyl-ether

(MTBE) from Sigma-ALDRICH (Steinheim, Germany). Carotenoid standards (98% pure) used for HPLC analysis were purchased from Extrasynthese (Genay, France): β-carotene, α + β-carotene mixture, lutein and β-apo-8-carotenal.

2.3. Carotenoids extraction and HPLC analysis of Musa-based dishes

Carotenoids extraction was carried out using protocols described in a previous study (Dhuique-Mayer, Tbatou, Carail, Caris-Veyrat, Dornier, & Amiot, 2007a). Samples of *Musa*-based dishes (0.5-2g) were extracted with ethanol/hexane (4:3, v/v). *β*-Apo-8'-carotenal was added as an internal standard. Carotenoids extracts were dissolved in 500 µL of dichloromethane and 500 µL of an 80:20 (v/v) mixture of MTBE and methanol before injection in HPLC. Carotenoids were analyzed by reverse-phase HPLC using an Agilent 1100 system (Massy, France), using a C_{30} column (250 x 4.6 mm i.d., 5 µm YMC (EUROP GMBH, Germany). The mobiles phases used were H_2O as eluent A, methanol as eluent B and MTBE as eluent C at a flow rate was fixed at 1 mL.min^{-1}, column temperature of 25°C, and an injection volume of 20 µL. The following gradient program was used: 0-5 min, 40% A, 60% B; 5-10 min, 20% A, 80% B (initial condition); 10-60 min, 4% A, 81% B, 15% C; 60-71 min, 4% A, 11% B, 85% C; 71-72 min 100% B, and back to the initial condition for re-equilibration. Absorbance was followed using an Agilent 1100 photodiode array detector. Quantification of carotenoids was achieved using calibration curves with β-carotene and lutein at 450 nm and at five concentration levels. Correlation coefficients ranged from 0.994 to 0.998.

2.4. Carotenoid extraction from digested samples

Carotenoid extraction was performed as described previously (Dhuique-Mayer, Borel, Reboul, Caporiccio, Besancon, & Amiot, 2007b). An aliquot of micellar aqueous fraction from a digested sample (10 mL) was extracted 3 times with 10 mL of hexane and 5 mL of ethanol containing 100 µL of β-apo-8'-carotenal as recovery standard. The pooled hexanic extracts were evaporated and redissolved in 500 µL of mobile phase (250 µL of dichloromethane and 250 µL of an 80:20 (v/v) mixture MTBE and methanol). Samples were injected according to analytical conditions as described above.

2.5. In vitro digestion

The *in vitro* digestion model was based on previous studies (Dhuique-Mayer, Borel, Reboul, Caporiccio, Besancon, & Amiot, 2007b; Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). However, an oral phase was added for the *Musa* samples in order to more closely simulate physiologic digestion. Triplicate samples (5 g each) of boiled bananas were subjected to simulated oral, gastric and small intestinal phases of digestion. In brief, samples were mixed with a saliva solution (6 mL) prepared by dissolving: 0.5208 g $NaHCO_3$ (99.5%), 0.0878 g NaCl (99.5%), 0.0478 g KCl (99.5%), 0.044 g $CaCl_2.H_2O$ (97%), 0.1044 g K_2HPO_4, 0.216 g mucin and 200 units/mL porcin α-amylase in 100 mL of ultrapure water, to obtain a solution that approximates physiologic conditions (Arvisenet, Billy, Poinot, Vigneau, Bertrand, & Prost, 2008). The pH was adjusted to 7.0 and the mixture was incubated for 10 min at 37°C in a shaking water bath. Samples (5 g for banana or dish samples and 10 g for porridge) were then mixed in saline solution (NaCl 0.9 %) and were stirred for 10 min at 37°C in a shaking water bath. To mimic the gastric digestion step, the pH was adjusted to 4.00 ± 0.02 with 1 M NaOH, after which 2 mL porcine pepsin (40 mg.mL^{-1} in 0.1 M HCl) was added. The homogenate was incubated at 37°C in a shaking water bath for 30 min. To mimic the intestinal digestion step, the pH of the partially digested mixture was raised to 6.00 ± 0.02 by adding 20 mL of 0.45 M sodium bicarbonate pH 6.0. Subsequently, 9 mL of a mixture containing 2 mg.mL^{-1} pancreatin and 12 mg.mL^{-1} bile extract in 100 mmol.L^{-1} trisodium citrate, pH 6.0, and 4 mL bile extract at 0.1g.mL^{-1} were added. Samples were incubated in a shaking water bath at 37°C for 30 min to complete the digestion process. Micelles were separated by centrifugation (20,000 rpm for 4 h at 10°C using a JA 21 rotor, model Avanti, J.E Beckman Coulter (USA) and the aqueous fraction was collected and filtered through a 0.22 µm filter (Millipore). Aliquots were stored at –20°C under nitrogen until analysis.

2.6. Vitamin A calculation

The vitamin A activity in the prepared banana dishes was calculated as Retinol Activity Equivalent (RAE) units, using the conversion factors: 12 µg.g^{-1} t-BC or 24 µg.g^{-1} t-AC carotenoids corresponding to 1 RAE.g^{-1} (Yeum & Russel, 2002). The daily vitamin A Recommended Dietary Allowances (RDA) for children under 6 years old is 400 RAE and the RDAs for a woman of reproductive age is 700 RAE (FAO/WHO, 2002). Using RAE, the mean total pVAC values derived from results of three independent HPLC analyses from each dish were converted into all-*trans* β-carotene equivalents (t-BCE).

2.7. Statistical analysis

Statistical analyses were performed using the XLSTAT software (version 11) and the statistical package for social sciences (SPSS) version 17. Analysis of variance (ANOVA) was used to test differences in mean values of *in vitro* bioaccessible carotenoids. Where ANOVA indicated significant differences between the treatments, Tukey's Honestly Significant Difference multiple rank test was used to further analyze these differences.

3. Results and discussion

3.1. Content of Provitamin A carotenoids (pVACs) in prepared bananas and Musa-*based dishes*

Carotenoids content in the boiled fruits and dishes derived from the plantain 'Musilongo' and the East African cooking banana 'Vulambya' were represented by three pVACs: all-*trans* β-carotene (t-BC), all-*trans* α-carotene (t-AC) and 13-*cis*- β-carotene (Table 2). Lutein was also detected but in lower amount or traces amounts. No significant differences between the carotenoids contents of *Musa* cultivars boiled with or without the fruit peel (p=0.05) were found. However, there were significant differences between the two cultivars with the boiled plantain' having higher levels of all three pVACs compared to the boiled AAA-EA 'Vulambya'. In all the 'Musilongo' boiled fruits, the proportion of t-BC was significantly higher (49%) than that of t-AC (37%). On the other hand, in all the boiled fruits from 'Vulambya', the proportion of t-BC was significantly lower (36%) than that of t-AC (56%).

Among the *Musa*-based dishes, 'Musilongo' porridge (T6) had the lowest content of both specific pVACs and total pVACs (0.07 $mg.kg^{-1}$). This low content can be explained by the double processing, first into flour involving fermentation, sun-drying and grinding, and then into porridge (Table 1-T5 & T6). In addition, the *Musa* flour constituted only about 8% of the ingredients used when preparing the porridge. The flour had a total pVACs' content of 3.33 mg.kg^{-1} compared with 75.93 mg.kg^{-1}dw observed in raw 'Musilongo', indicating that there are already almost no pVACs left in the flour, even before preparation of the porridge.

Other ingredients used in the dishes included fresh beans, amaranth leaves and occasionally the addition of either olive or palm oil during the boiling process. The inclusion of olive oil or beans did not alter the overall carotenoids content, which was to be expected as the analysis of the single ingredients had shown that neither olive oil nor beans contain any carotenoids. In contrast, the addition of amaranth leaves (Table 1B-T12 & T13) led to an increase in the content of t-BC in the 'Vulambya' dish (6.68 mg.kg^{-1} compared to 0.80 mg.kg^{-1}). This was due to the reported high level of t-BC (approximately 1725 mg.kg^{-1} edible portion) in amaranth leaves (Grubben and Denton, 2004). Similar trends were observed with the addition of palm oil. The content of t-BC following addition of palm oil during boiling of the two banana cultivars was significantly increased by 2.5 and 9 fold respectively for 'Musilongo' and 'Vulambya'. This is because Palm oil is well known for it's high level of t-BC content which ranges between 580-2390 mg.kg^{-1} (Monde, Michel, Carbonneau, Tiahou & Vernet, 2009).

3.2. *Contribution of the* Musa-*based dishes to daily vitamin A Recommended Dietary Allowances*

The estimated RAE of the *Musa*-based dishes and their potential to contribute to meeting the nutritional requirements of children below five years and women of reproductive age are reported in Table 2. Foods with an RAE of around 70 or higher are considered to be a good source of vitamin A (Englberger et al., 2006). 'Musilongo' porridge, with its very low levels of pVACs, could not significantly meet the RDAs for vitamin A. On the other hand, boiled

'Vulambya' and 'Musilongo', had RAE levels ranging from 19.2 to 22.0 µg.100g^{-1} and from 72.9 to 73.4 µg.100g^{-1}, respectively. According to Rodrigues-Amaya (1997), consumption of between 200-500g of boiled *Musa* fruit by a child 1-5years and a woman of reproductive age is considered to be within normal levels. Therefore with these consumption levels, boiled 'Musilongo' would meet between 36% to 52% of the vitamin A RDAs of a child 1-5years and a woman of reproductive age. Consumption of the same quantities of boiled 'Vulambya' would only meet 10 to11% and 14 to16% of the vitamin A RDA needed by the same child and the same woman, respectively. The addition of beans, containing no carotenoids, does not increase the contribution to RDA for vitamin A, but dishes derived from boiled 'Musilongo' and 'Vulambya' with pVAC-rich amaranth leaves and/or palm oil, on the other hand, could significantly contribute to daily vitamin A requirements. Within the normal consumption levels stated earlier, 'Vulambya' boiled with fresh beans, amaranth leaves and olive oil would meet 44.3% and 63.3% of the vitamin A RDA of a child of 1-5 years and a woman of reproductive age, respectively, while consumption of 'Musilongo' boiled with or without olive oil would meet 28.8-36.7% and 41.1-52.4 % of the vitamin A RDA of the same child and the same woman, respectively (Table 2).

3.3. Bioaccessibility of pVACs from prepared Musa*-based dishes*

The percentage of micellarization of t-BC, t-AC, and 13-*cis*-BC are reported in Table 3. The micellarization of t-BC was significantly more efficient in the boiled 'Vulambya' (28.9%) compared with boiled 'Musilongo' (16.6%). However, when taking into account the lower initial levels of pVACs, 'Vulambya' represented a lower final Retinol Equivalent (22.4 µg.200g^{-1}) compared with 'Musilongo' (48.9 µg.200g^{-1}). These values are high compared to other starchy foods rich in carotenoids, such as orange fleshed sweet potato whose t-BC bioaccessibility has been reported to be between 0.6 and 3% (Failla, Thakkar & Kim, 2009). With respect to the different carotenoid species, the bioaccessibility of t-BC and t-AC were similar (29 and 17% for t-BC, and 31 and 16% for t-AC, respectively in boiled ’Vulambya’ and ‘Musilongo’), while that of 13-*cis* BC was higher (33 and 21%, respectively). Similar observations were previously reported by several authors for other food types (Failla, Thakkar & Kim, 2009; Bengtsson, Larsson-Alminger & Svanberg, 2009; Reboul, Richelle, Perrot, Desmoulins-Malezet, Pirisi, & Borel, 2006). The inclusion of an oral phase with artificial salivary in this *in vitro* digestion model had no effect on the bioaccessibility of pVACs from boiled bananas (p=0.05).

In our study, t-BC and t-AC from porridge made from ‘Musilongo’ flour were more bioaccessible than those from boiled ‘Musilongo’. The difference in bioaccessibility could be explained by the fact that the liquid state of the food (moisture content 93 %) could help the micellarization of carotenoids present. However, despite the relatively high bioaccessibility, the negligible initial levels of pVACs in ‘Musilongo’ porridge mean that this food is a very poor source of vitamin A.

The addition of olive oil, when boiling ‘Musilongo’, did not significantly increase the bioaccessibility of pVACs. This could be explained by the preparation method, where oil was added to the water during the boiling process and the banana fruits were subsequently removed from the oil and water mixture. As such, the oil can be assumed to have been discarded with the cooking water and was hence not incorporated into the food. Similar results with respect to bioaccessibility were observed when palm oil was added to boiled ‘Musilongo’ (no increase in bioaccessibility), with the difference that the initial pVACs content was greatly increased, resulting in a Retinol Equivalent of 86 µg providing 21.6% of the RDA for children.

In the dishes derived from 'Vulambya', the pVACs bioaccessibility increased slightly in the presence of olive and palm oil. In contrast to the above, here oil was better incorporated to the banana/beans mixture, probably improving micellarization of carotenoids. Moreover, the high moisture content (70%) and the softness of the fruit of the AAA-EA cultivar 'Vulambya' as compared to the plantain (Amankwah, Ayim, Dzisi & Barimah, 2011) led to a better incorporation of oil during cooking. As expected, the addition of local red palm oil increased the carotenoids content of the meal and provided around 28% of the RDA for children. When amaranth leaves were added to the 'Vulambya' and beans dish, the bioaccessibility particularly for β-carotene decreased from 24 to 10.2. This was explained by the fact that the majority of t-BC came from amaranth leaves, 6.7 mg.kg^{-1} compared to 0.8 mg.kg^{-1} observed in the 'Vulambya' and bean dish (Table 2). It is well known that the bioavailability of β-carotene is higher for fruits as compared to green leafy vegetables because of the localization of carotenoids in the food matrix. (Van Het Hof, West, Weststrate & Hautvast, 2000; De Pee, West, Permeisih, Martuti & Hautvast, 1998). In green leafy vegetables, carotenoids are found in chloroplasts bound to protein and fiber and the release from the food matrix is difficult, in fruits carotenoids are in chromoplasts dissolved in oil droplets (O'Connell, Ryan & O'Brien, 2007). In the 'Vulambya'-bean-amaranth dish, the main β-carotene from amaranth leaves resulted in a lower bioaccessibility of carotenoids and the addition of olive oil slightly improved the bioaccessibility of β-carotene.

Amongst the different *Musa*-based dishes analyzed, the two dishes with palm oil (plantain 'Musilongo' boiled without the fruit peel and palm oil, and the AAA-EA 'Vulambya' boiled with beans and local palm oil) were the most interesting in terms of their potential to contribute to meeting the RDA of target populations. These were followed by 'Vulambya' boiled with beans, amaranth leaves and olive oil, and boiled 'Musilongo'. Taking bioaccessibility into account and applying a 50% conversion of BC to retinol (Yeum & Russel, 2002), this study has indicated that a 200 g portion of boiled peeled 'Musilongo' with local palm oil could provide 22% of the daily vitamin A requirement of a child 1-5 years; 200 g of 'Vulambya' boiled with beans and palm oil would provide 28%. In comparison, a 200 g portion of boiled 'Musilongo' without palm oil would only provide 12%.

Estimation of the vitamin A activity in *Musa*-based dishes calculated from an undigested food using a classical estimate (RAE) or calculated from a digested food taking into account bioaccessibility (RE), lead to different results (Figure 1). Classical estimates from undigested food overestimated RAE compared with estimates from digested food. These last results suggest that it is very important to take into account the t-BC bioaccessibility of *Musa*-based dishes in carotenoids absorption and consequently in estimations of the vitamin A activity to meet nutritional requirements.

When 'Musilongo' or 'Vulambya' was boiled as a single ingredient without addition of other ingredients such as oil and vegetables, the pVACs contents of the dish were not sufficient to provide the daily vitamin A requirement of a child of less than 6 years. While we assessed the most popular AAA-EA and plantain cultivar in the region under study, the population depends on a much greater diversity of banana and plantain cultivars. More studies are hence needed to provide a more detailed picture of the pVACs levels and bioaccessibility in this wider range of cultivars that are consumed in Eastern DR Congo. Among several factors that might influence the effectiveness of a certain food crop to prevent vitamin A deficiency, varietal differential pVAC levels could be the most important as reported by Burri (2011) for sweet potato.

4. Conclusions

In conclusion, the bioaccessibility of pVACs from boiled bananas depended on the cultivar, and ranged from 10 to 32%. These values are high compared to other starchy foods rich in carotenoids, such as orange fleshed sweet potato. Our results also show that addition of ingredients known to be rich in pVACs to the bananas during boiling strongly influences the bioaccessibility of pVACs. Consequently, the classical estimates of RAE from a food could often represent an overestimation of vitamin A contents. This study further highlights the need to determine pVACs bioaccessibility as a critical step when establishing the vitamin A activity of a particular food product or dish, as we saw that the classical estimates of retinol activity equivalent from a food may overestimate the actual retinol equivalent.While more investigations are needed to evaluate carotenoids bioaccessibility from different *Musa* cultivars and dishes, these data support the promotion/consumption of *Musa*-based food to help reduce vitamin A deficiency in African countries, but highlight the importance of cultivar and processing choice on the final potential to contribute to meeting the RDA of target populations

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Figure caption.

Figure 1. Retinal equivalent expressed in RAE[1] or RE[2] per 100g in the freshly prepared and digested *Musa* products. [1] RAE: retinol activity equivalent= µg *trans* β-carotene /12 and 13 *cis*-β-carotene /24; [2] RE : retinol equivalent = µg trans β-carotene /6 and 13 *cis*- β-carotene /12

Figure 1

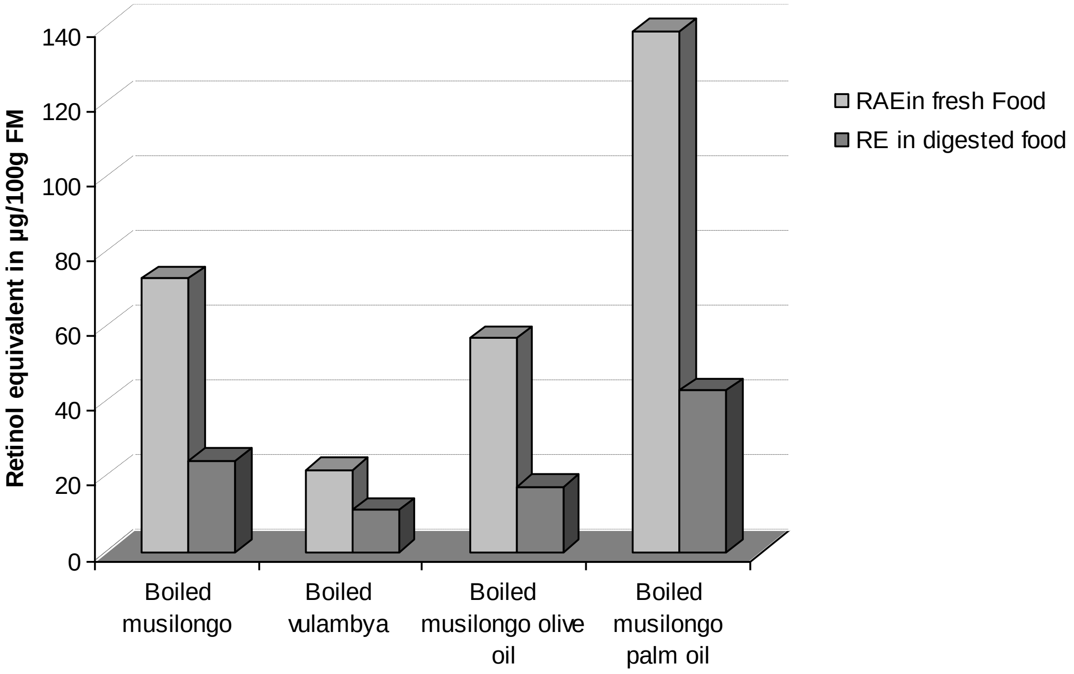
140
120
100
80
60
40
20
0
Retinol equivalent in µg/100g FM
RAEin fresh Food
RE in digested food
Boiled musilongo
Boiled vulambya
Boiled musilongo olive oil
Boiled musilongo palm oil

Table 1A. Ingredients and procedures used by community members in preparation of the *Musa* dishes ('Musilongo').

	***Musa* Product**	**Ingredients**			**Cooking procedure**
		Musa Fruit	**water**	**oil**	
T1	Musilongo boiled in peel	3 fingers unpeeled 480 g	300 ml	-	The fingers were cleaned, weighed and placed in an a sauce pan. Water was added to just submerge the fruits. These were boiled, covered, at medium heat for 15 minutes. The remaining water was drained, and the fruits were cooled with the lid on. The boiled fruits were then hand peeled, weighed and placed in labeled zip-locked plastic bags with air removed manually and stored at -20^{0}C .
T2	Musilongo boiled without peel	3 fingers hand peeled 340 g	300 ml	-	Idem[1] as T1, but fruits were hand-peeled after cleaning and before weighing.
T3	Musilongo boiled without peel with olive oil	3 fingers hand peeled 340 g	300 ml	10g (2.9%)	Idem as T2, but 1 table spoon of olive oil was added during boiling.
T4	Musilongo boiled without peel with palm oil	3 fingers hand peeled 340 g	300 ml	10g (2.9%)	Idem as T2, but 1 table spoon of palm oil was added during boiling.
T5	Musilongo flour	3 Unripe Musilongo fingers	-	-	Mature unripe Musilongo fingers were peeled using a knife, put in polythene bags which were closed tightly and left to ferment for 3 days. The mould was scrapped off the finger using a knife and the bananas were put under the sun for 1-2 days to dry. Once dry, they were crushed into small pieces and ground into flour using a local grinding mill. Note: This is the flour used in making the porridge (T6)
T6	Musilongo porridge	30g Musilongo flour,	350 ml	-	300ml of water was put in a sauce pan and let to boil, when it boiled, heat was reduced. 30g of Musilongo flour was put in a cup and 50ml of water added, this mixture was stirred well until smooth and after thick and stable. It was then let to boil for 2minutes before being let to cool. The cooled porridge was poured into a clean container with a lid and stored at -20°C.

[1] Idem : the same

Table 1B. Ingredients and procedures used by community members in preparation of the *Musa* dishes ('Vulambya').

	***Musa* Product**	**Ingredients**					**Cooking procedure**
		Musa fruit	**beans**	**amaranth**	**water**	**oil**	
T7	Vulambya boiled in peel	3 fingers Unpeeled 360 g		-	300 ml	-	Idem T1.
T8	Vulambya boiled without peel	3 fingers hand peeled 200 g		-	200 ml	-	Idem as T7, but fruits were carefully peeled with a knife after cleaning and before weighing. A pinch of salt was added to the water Before starting to cook.
T9	Vulambya boiled with beans	3 fingers hand peeled 200g	boiled fresh beans 100 g	-	500 ml	-	The fresh beans were sorted and put to boil. When they were soft (cooked), peeled and cleaned banana fingers were added to the beans, water added and salt to taste. The mixture was covered and let to cook for about 15 minutes under medium heat until the bananas were cooked. After cooking, the dish was let to cool and put in labeled zip-lock bags with air removed manually and stored in a dark rook -20^{0}C.
T10	Vulambya boiled with beans and olive oil	3 fingers hand peeled 200g	boiled fresh beans 100 g	-	500 ml	10 g (3.3%)	Idem as T9, but when both the bananas and beans were cooked (15minutes), 1 table spoon of olive oil was added to the mixture; the mixture was stirred a little and left to cook for about 2 more minutes.
T11	Vulambya boiled with beans and palm oil	3 fingers hand peeled 200g	boiled fresh beans 100 g	-	500 ml	10 g (3.3%)	Idem as T10,
T12	Vulambya boiled with beans and green vegetables	3 fingers hand peeled 200 g		amaranth leaves, 60 g	600 ml		The fresh beans were sorted and put to boil, when they were soft (cooked), peeled and cleaned banana fingers were added in the beans, water added and salt to taste. The mixture was covered and let to cook for about 12 minutes under medium heat. Amaranth leaves were added after the 12 minutes, the mixture was mixed a little bit and let to cook for 5 more minutes before being left to cool with lid on. The dish was put in labeled zip-lock bags with air removed manually and stored at -20^{0} C .
T13	Vulambya boiled beans amaranth olive oil	3 fingers hand peeled 200 g	boiled fresh beans 100 g	amaranth leaves, 60 g	600 ml	20 g (2.8%)	Idem[2] as T12, but 2 table spoons of olive oil were added were added to the bananas, beans and amaranths, and mixed in well just 2 minutes before the dish was removed from the heat.[2]

Table 2. Content of pVACs in freshly processed *Musa* products and *Musa*-based dishes consumed in Eastern Democratic Republic of Congo

Mush product/Dish	Content of pVACs[1] (mg/kg) in freshly prepared Products and *Musa* dishes									
	All-*trans*-β-carotene		α-carotene		13 *cis*-β-carotene		Total	RAE in	RDA child	RDA-FA
	Mean (ratio)	SD	Mean(ratio)	SD	Mean(ratio)	SD	pVAC	μg/100g	200g/day	500g/day
Musilongo boiled in peel	5.75(49.4%)	0.09	4.27(36.7%)	0.44	1.62(13.9%)	0.05	11.64	72.9	36.5%	52.1%
Musilongo boiled without peel	5.93(51.3%)	0.06	4.03(34.9%)	0.12	1.59(13.8%)	0.03	11.55	73.4	36.7%	52.4%
Vulambya boiled in peel	1.39 (35.6%)	0.14	2.19 (56.2%)	0.07	0.32 (8.2%)	0.02	3.9	22	11.0%	15.7%
Vulambya boiled without peel	1.19 (35.8%)	0.01	1.85 (55.7%)	0.06	0.28 (8.4%)	0.01	3.32	19.2	9.6%	13.7%
Musilongo flour	1.76 (52%)	0.08	1.42 (46%)	0.07	0.15(4.5 %)	0.04	3.33	-	-	-
Musilongo porridge	0.07 (53.8%)	0.01	0.06 (46.2%)	0.01	0.00 (00.0%)	0	0.13	0.8	0.4%	0.6%
Musilongo boiled olive oil	4.51 (48.7%)	0.21	3.36 (36.3%)	0.16	1.39 (15.0%)	0.05	9.26	57.6	28.3%	41.1%
Musilongo boiled palm oil	11.5 (52%)	0.3	7.22 (32.6%)	0.15	3.36 (15.2%)	0.03	22.08	139	69 .5 %	99.0%
Vulambya, Beans	0.80 (33.5%)	0.05	1.41 (59.0%)	0.16	0.18 (07.5%)	0.02	2.39	13.4	6.7%	9.6%
Vulambya, beans, olive oil	0.71(33.2%)	0.01	1.10 (51.4%)	0	0.33 (15.4%)	0.04	2.14	11.9	5.9%	8.6%
Vulambya, beans, palm oil	7.45 (55.6 %)	0.13	4.40 (32.8 %)	0.07	1.54 (11.5)	0.05	13.39	86.8	43.4	62.0%
Vulambya, beans, amaranth	6.68 (62.4%)	0.15	2.26 (21.1%)	0.35	1.77 (16.5%)	0.04	10.71	72.8	36.4%	52.0%
Vulambya, beans, amaranth, olive oil	8.62 (68.4%)	0.09	2.47 (19.6%)	0.07	1.51(12.0%)	0.03	12.6	88.7	44.3%	63.3%[1]3

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[3] Values are means of three independent determinations (percentage proportion of the specific carotenoids in relation to total provitamin A carotenoids), SD=standard deviation. RAE= Retinal Activity Equivalent = μg trans β-carotene /12 and 13 *cis*- β-carotene /24. FA=Female Adult. ($p=0.05$)

Table 3. Bioaccessibility of pVACs in freshly processed *Musa* products and dishes consumed in Beni territory, Eastern Democratic Republic of Congo

Bioaccessibility of pVACs (%) in *Musa* products and dishes	β-Carotene	α-carotene	13 *cis* β-Carotene	µg RE* /200g after in vitro digestion	%RDA child < 6 years (to meet 400RE)
	% Bioac (SD)	% Bioac (SD)	% Bioac (SD)		
Musilongo no oral phase	16.6 (2.7) a	15.5 (1.8) a	21.5 (1.3) a	48.88	12.2
Musilongo with oral phase	14.1 (0.4) a	15.2 (1.0) a	23 (1) a	-	-
Vulambya no oral phase	28.9 (1.0) b	30.5 (0.8) be	33 (2) b	22.38	7
Vulambya with oral phase	28.7 (1.8) b	28.6 (1.2) be	33.5 (1.2) b	-	-
Musilongo porridge	31 (1) bd	41 (1) c	nd	-	-
Musilongo olive oil	14.6 (2.1) a	14 (1.8) a	23.7 (2.0) a	35.2	8.8
Musilongo palm oil	15.3 (1.8) a	13.8 (1.6) a	20 (2) a	86.44	21.6
Vulambya and Beans	24 (2)c	23.5 (2.5) d	16.2 (2.2) c	12.4	3.1
Vulambya, beans, olive oil	27.5 (3.3)bc	26(3.6) e	nd	5.63	1.4
Vulambya, beans, palm oil	32 (3)d	31.1(3.3) b	34.4 (4.2) b	110.08	27.7
Vulambya, beans, Amaranth	10.2 (0.2)e	22.7 (1.6) d	21.6 (1.3) a	38.96	9.7
Vulambya, beans, amaranth, olive oil	15.8 (1.9) a	23.7 (2.7) d	22 (2.1) a	60.66	15[4]

[1] * RE= retinol equivalent = µg *trans* β-carotene /6 and 13 *cis*-β-carotene /12; n≥3 independent experiments presented as means (SD); nd, not detected; Significant differences in the same column are shown by different letters ($p<0.05$)

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[4]