Vol. 11(3), pp. 68-79, March 2019
DOI: 10.5897/JPBCS2018.0776
Article Number: 17B0C6760373
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

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Journal of Plant Breeding and Crop Science

Review

Advances in carotenoid increments in storage parts of African staple crops

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Received 23 September, 2018; Accepted 12 November, 2018

The importance of vitamin A and other carotenoids in controlling micronutrient based deficiencies in particular VAD has been emphasized in recent years. This has resulted into demands for availing these nutrients in forms that are easily accessible for most of the populations in micronutrient deficient areas. Specifically in Africa, various programs have been instituted to bio-fortify crops with nutrients with more emphasis being put on Vitamin A fortification. Much as advances have been made in this area, a number of programs have registered little success while others have not taken off. In this review, advances in breeding for vitamin A increments are discussed. Countries where successes have been achieved are also highlighted while efforts in a number of areas for the different staple crops have been given due emphasis. In particular, breeding strategies have been discussed, and examples of successful breeding strategies highlighted to inform future efforts. In addition, the effect of processing on retention of vitamin A in processed products has been discussed with specific recommendations on identification of crop specific processing procedures. Such procedures should be optimized before adoption to allow for minimal losses in vitamin A and other related nutrients. We conclude that much as advances have been made, specific efforts are still needed in certain staples in order to provide benefits to the African consumers.

Key Words: African staples, Carotenoids, Malnutrition, Biofortification, Retention

INTRODUCTION

Vitamin A (retinol) is essential for vision, cell growth and tissue differentiation, and is critical for development during pregnancy and breastfeeding. Preformed vitamin A is found almost exclusively in animal products. Vitamin A content ranges from about 30 µg retinol per 100 ml in full cream milk up to as much as 16,000 µg of retinol per 100 g in the liver (Wijesundera et al., 2012). However, dependence on animal products is not sustainable in sub-Saharan Africa as such products are not affordable and are associated with major chronic syndromes such as cardio-vascular disease. As a result, there is need to provide precursors for vitamin A in adequate amounts in most staple crops. Carotenoids, especially α and β-carotene, which are potential vitamin A precursors are present in plants and plant products and some may contain up to 310 µg of β-carotene per gram especially in

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fruits and vegetables (Khoo et al., 2011). Carotenoids are biologically less available than retinol but highly abundant in plant sources than animal foods. However, fruits and vegetables are seasonal crops with variable availability (FIT-Uganda, 2006) and hence daily supply cannot be guaranteed as the consumption patterns of fruits and vegetables are uncertain. For instance, it was reported in Cincinnati city of Ohio that only 18% of adults eat the recommended daily serving of fruit or vegetable (Interact for Health, 2014). In a related study in Minnesota, USA, it was reported that 91% of males and 86% of females consumed less than 3 servings of fruit or vegetable per day despite reported availability by 89% of the subjects (Arcan et al., 2007). Similarly, certain population groups (prisoners and refugees) take restricted diets that exclude certain types of micronutrient rich foods. Such consumption patterns show that there is need for alternative measures for meeting vitamin A requirements in various populations.

Carotenoids (vitamin A precursors) are C40 polyenes which are essential in plant development, photosynthesis, root mycorrhizal interactions and production of phytohormones (Esuma, 2016). Carotenoids comprise a large isoprenoid family and most are 40-carbon tetra-terpenoids derived from phytoene (DellaPenna and Pogson, 2006). The carotenoid backbone is either linear or contains one or more cyclic β-ionone or ε-ionone rings or, less frequently, the unusual cyclopentane ring of capsanthin and capsorubin (Arimboor et al., 2015). Carotenoids exist in non-oxygenated forms referred to as carotenes or their oxygenated derivatives the xanthophylls (DellaPenna and Pogson, 2006). The most commonly occurring carotenes are β-carotene, stored in chloroplasts and lycopene in chromoplasts of some flowers and fruits and other plant storage organs. The most abundant xanthophylls (lutein, violaxanthin, and neoxanthin) occur in photosynthetic plant tissues as vital components of the light-harvesting complexes (DellaPenna and Pogson, 2006).

Carotenoid biosynthesis and storage in plants

Carotenoid pigments are produced by the isoprenoid biosynthesis pathway and uses isopentenyl pyrophosphate (IPP), a 5-carbon compound, as the building block. There are two biosynthetic routes for IPP biosynthesis: a) the major one occurs in the cytoplasm and utilizes acetyl-CoA units in sequential reactions that lead to formation of IPP; b) the second route occurs in the chloroplasts, whereby, IPP is formed through a reaction initiated by the condensation of pyruvate and glyceraldehyde-3-phosphate (G-3-P). This reaction is catalyzed by enzyme 1-deoxy-D-xylulose-5-phosphate synthase and leads to the formation of 1-deoxy-d-xylulose 5-phosphate (DXP). The DXP reductively isomerizes to form a 2C-methyl-d-erythritol-2, 4-cyclophosphate (MEP) in a reaction catalyzed by enzyme DXP reducto-isomerase. Finally, MEP is subsequently converted through a series of reactions to isopentyl-pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP).

Thereafter, various isoprenoids can be formed through condensation of various units of IPP molecules. Typically, a molecule of IPP condenses with one molecule of DMAPP to form geranyl pyrophosphate (GPP) in a reaction catalyzed by GPP synthase. The next steps involve sequential addition of IPP molecules to form a 20-carbon compound, geranyl geranyl pyrophosphate (GGPP) (Figure 1). Two molecules of GGPP condense to form phytoene, in a reaction catalyzed by phytoene synthase (PSY) (Cunningham Jr and Gantt, 1998). Phytoene then undergoes a series of four desaturation reactions resulting into formation of lycopene (ψ, ψ -carotene). Lycopene is vital in synthesis of carotenoid compounds in plants.

Typically, lycopene is cyclized to form α-carotene and β-carotene, in a reaction catalyzed by enzyme lycopene β-cyclase (LCYB). The α-carotene can be further oxidized to form zeinoxanthin and leutin, while β-carotene can be oxidized to form either zeaxanthin, or violaxanthin, or neoxanthin, depending on the plant species (Cunningham Jr and Gantt, 1998; DellaPenna and Pogson, 2006). The carotenoids formed in synthetic cells especially in leaves can then be taken from the source organs to the sink via protein mediated translocative activities that are tightly regulated to reduce photo-oxidation. In staple crops, storage of such carotenoids is essential as a micronutrient to populations that depend on such staples. These provitamin A carotenoids from plants represent an additional major dietary source of vitamin A for most of the world's population (Weber and Grune, 2012).

Micronutrient malnutrition and Vitamin A deficiency (VAD)

Micronutrient malnutrition is a major underlying cause of health problems in developing countries. In particular, Vitamin A deficiency (VAD) can result either into night blindness or *Xerophthalmia* (dryness of the conjunctiva and cornea) and *Keratomalacia* (softening and ulceration of the cornea); causing total blindness (Semba, 1998; Gegios et al., 2010). The deficiency is more prevalent in children under 5 (Maziya-Dixon et al., 2006) and in pregnant mothers due to high nutrient demands by the fetus and mother. VAD accounts for over 600,000 deaths each year globally among children below 5 years of age (Hotz et al., 2012). Indeed, the World Health Organization (WHO) classified VAD as a public health problem in one third of children aged 6 months to 5 years in 2013. Of these, the highest rates were in sub-Saharan Africa at 48% and South Asia at 44% (Stevens et al., 2015; UNICEF,

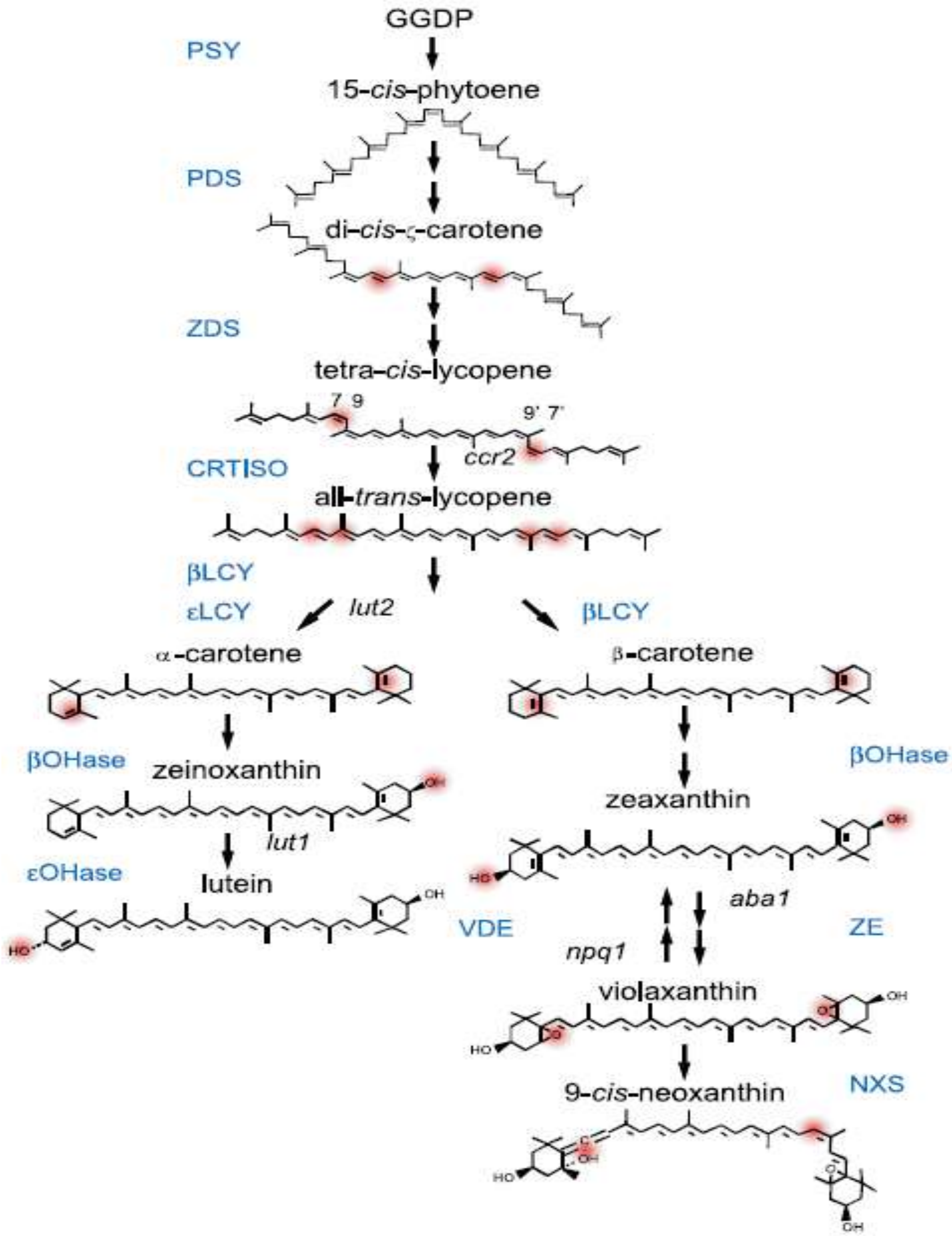

Figure 1. The biosynthetic process for carotenoids in plants: The pathway for carotenoids in plants. Adapted from (DellaPenna and Pogson, 2006). CRTISO, Carotenoid isomerase; β-LCY, β-carotene cyclase; βOHase, β-carotene hydroxylase; εLCY, ε-cyclase; εOHase, ε-carotene hydroxylase; NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; VDE, violaxanthin deepoxidase; ZDS, ζ-carotene desaturase; ZE, zeaxanthin epoxidase.

2016).

Beyond the role in vision, vitamin A plays a critical role in modulation of the immune function of the body. Notably, experimental observations in early 1920s and 30s led to the reputation of vitamin A as the "anti-infective" vitamin (Semba, 1998) which on supplementation reduced child mortality by 30% (Mora et al., 2008). Indeed, high-dose vitamin A supplementation had become recommended therapy for measles in developing countries and in the United States in the 1980s (Semba, 1998). Supplementation with vitamin A has also been shown to enhance resilience in AIDS patients by increasing CD4^{+} lymphocytes during HIV infection (Semba, 1998) and enhances immunity against cancer and HIV (Semba, 1998; Mora et al., 2008).

FORTIFICATION OF STAPLE FOODS FOR PROVITAMIN A ENHANCEMENT

Food fortification refers to addition of an essential nutrient to a food (Allen et al., 2006). The success of any food-fortification programme is the improvement in the nutritional and health status of a targeted population (Wirakartakusumah and Hariyadi, 1998). In line with this, the fortified food should be accepted and consumed by the targeted population. Thus, factors such as quality, taste, and cost of the fortified products play important roles in determining the effectiveness of the fortification programmes. There are two approaches of food fortification: conventional fortification, involving addition of nutrients during food processing, and biofortification, where a plant carrier is modified to express the added nutrient during growth.

Conventional food fortification

Conventional food fortification is the addition of essential nutrients to a food during food processing in appropriate concentrations that ensure accuracy and consistency (Organization, 2016). This depends on a well-functioning dosing technology and a reliable method of detection. The process involves the use of a food carrier (food to be fortified) to which the target nutrients are added either singly or in a premix (cocktail of target nutrients). Following the fortification process, detection of the added nutrient is carried out to confirm presence of the nutrient, in the desired quantities.

Conventional food fortification is particularly effective in tackling deficiencies, especially in densely populated urban areas where land for cultivation of food crops is scarce (Triggle, 2004). This method of nutrient enhancement is also attractive because it does not require the target groups to change their diet but can be implemented by the food industry and because it reaches large numbers of consumers through retail (Triggle, 2004). World Bank studies suggested that the annual per capita cost of fortifying a food with vitamin A is between USD 0.69 USD and USD 0.98 per capita per year (Triggle, 2004). Conventional fortification would therefore be a more cost-effective method for impacting on vitamin A intake of communities, especially in urban areas.

However, the implementation and effectiveness of this method to alleviate vitamin A deficiency in populations that depend on own-saved food is difficult. Such programs are affected by other socio-economic factors that influence dependence on major staple foods. In particular, the subsistence form of agriculture, coupled with the pressing socio-economic demands such as education and health care compel the resource-poor farmers to sell off fruits, animals and animal products such as eggs in order to earn a living. Moreover, the cost of fortified foods is high and usually unaffordable for such farmers due to high poverty levels (Esuma, 2016; Iannotti et al., 2013). Therefore, there is always need to search for alternative dietary intervention methods so as to have a wider coverage, especially in vulnerable groups in rural areas.

Biofortification of staple food crops with carotenoids

Biofortification refers to the breeding and genetic modification of plants so as to improve their carotenoid content. It differs from conventional fortification in that biofortification increases nutrient levels in consumable crop storage organs during plant growth, rather than during processing of the crops (Organization, 2016). In this regard, the biofortified crop should be able to express the carotenoids during its growth period. Biofortification can be either by genetic engineering, normally referred to as modern biotechnology, or by conventional plant breeding.

Genetic engineering approaches have been used to either increase or modify the carotenoid content in plants through manipulations in the carotenoids biosynthetic pathway. Phytoene synthase (PSY) is a branching enzyme that directs substrates irreversibly to carotenoids. Hence, it has been the target in several genetic manipulation studies (Naik et al., 2003). For instance, the constitutive over-expression of PSY in plants that do not normally produce carotenoid pigments; as in tobacco, tomato and rice, led to substantial increase in carotenoid accumulation (Naik et al., 2003; Fraser et al., 2002; Paine et al., 2005). Similarly, the genetic manipulation of rapeseeds (*Brassica napus*) using a bacterial PSY gene (*crtB*) to increase carotenoid content resulted in up to a 50-fold increase in carotenoids, α- and β-carotenes being the predominant ones. Similarly, the PSY gene was also cloned into rice (Burkhardt et al., 1997; Ye et al., 2000) to induce carotenoids synthesis and storage, the case of the Golden Rice. In addition, DNA constructs aimed at upregulating the expression of lycopene β-cyclase gene were introduced in tomatoes and transformants showed significant increments in carotenoids content (Naik et al., 2003).

Conventional plant breeding refers to the crossing of plants with relevant characteristics, to form a crossbreed that exhibits inherited traits from both parent plants (Royal Society, 2016). This is followed by selection and multiplication of the offspring with the desired combination of characteristics. Depending on the crop, the conventional breeding process may take 10 or more years before a variety can be released to the grower (Caligari and Forster, 2012) and involves step-wise procedures leading to variety release (Fukuda and Saad, 2001; Esuma, 2016). Usually, farmers are involved in the final stages, especially at farm level testing (Esuma, 2016). Modified and shorter breeding procedures have been made for various crops (Kawuki et al., 2011; Pfeiffer and McClafferty, 2007) by modifying and/or eliminating specific steps in the conventional breeding process or by adopting molecular selection methods such as marker-assisted selection, marker-assisted recurrent selection, or marker-assisted back-crossing (Moose and Mumm, 2008). Other efforts such as the double haploid technology (DH) with potential improvement to the conventional schemes are also being developed. Currently, breeders are optimizing genomic selection tools using whole-genome coverage markers such as single nucleotide polymorphism (SNP) to develop prediction models which enormously reduce the breeding cycle and the number of hybrids to be evaluated in the field (de Oliveira et al., 2012).

Biofortification is advantageous in addressing micro-nutrient deficiency due to its long-term cost-effectiveness and its ability to reach underserved, rural populations. The upfront investments in plant breeding yields micronutrient-rich biofortified planting material for farmers to grow at virtually zero marginal cost. These can be evaluated and adapted to new environments, multiplying the benefits of the initial investment with minimal recurrent expenditures (Bouis and Saltzman, 2017). Biofortified crops can easily reach rural populations who have limited access to diverse diets or other micronutrient interventions. Target micronutrient levels for biofortified crops are set to meet the specific dietary needs of women and children, based on existing consumption patterns (Bouis and Saltzman, 2017; Mwanga et al., 2016). Subsequently, biofortification may present a way to reach populations with preferred food traits especially where supplementation/conventional fortification activities may be limited (Organization, 2016). Accordingly, global efforts have been tailored to biofortification of staple food crops to help alleviate deficiencies associated with overdependence on the foods. Major foods under biofortification programmes in Africa include sweet potato, cassava, maize, and banana.

CURRENT STRATEGIES FOR INCREASING CAROTENOID CONTENTS IN STORAGE PARTS OF THE PLANT

The elucidation of carotenoid biosynthesis pathway and genes involved in the control and regulation of the pathway is important in breeding for increased carotenoid content. This process can be hampered by: 1) the fact that synthesis of β-carotene is induced by GGPP, a metabolic precursor for other vitamins and pigments whose synthesis could be decreased; 2) interference with the well-balanced regulatory mechanism of the pathway and 3) the need for highly lipophilic nature of carotenoids provision of storage in plants. Therefore, high carotenoid production should focus on increased precursor supply, maintaining the balance between interacting metabolic pathways and targeting of tissues that are capable of incorporating lipophilic molecules (Naik et al., 2003). Also, increased levels of carotenoids in storage parts of higher plants might be due to down-regulation metabolite synthesis. Even then, efforts have been put forward to breed and increase provitamin A content in storage parts of major staple food crops in Africa (Table 1 and Figure 2).

Plant breeding has mainly been used to increase the carotenoid content in edible tissues of crops including non-photosynthetic storage parts. It has been observed that such processes do not cause any interruption to the plant, and that the pro-vitamin A levels can be influenced to attain the required thresholds. Once such plant varieties have been attained, rigorous promotion and awareness creation is then required for adoption. Such success has been attained with the Orange Sweet Potato (OSP) in tropical and subtropical Africa and can be easily applied to other African staples such as cassava, maize and bananas that are nutritionally superior, well adapted to local growing conditions and more profitable for farmers. However, the bioavailability of plant provitamin A varies widely in relation to the food crop, genotype (including sink activity of the storage organ) cooking method, individual genetic factors and consumption of fat with the meal. Other factors such as post-harvest food storage, processing environment and general food handling also affect carotenoid availability. Thus, optimisation of such factors for increased bio-availability is of paramount importance.

Breeding strategies for enrichment of Vitamin A in storage parts staple crops

Vitamin A enhancement in storage parts of staple crops has been achieved through a range of breeding approaches including transgenic, agronomic and conventional procedures. In the case of carotenoids, enrichment within the plant has been achieved through expression or up regulation of genes and or gene products involved in carotenoid synthesis (DellaPenna and Pogson, 2006). In a number of instances as related to transgenesis, genes can be added, removed or altered in such a way that the production and hence translocation of carotenoids from source to sink organs is enhanced. However, in all cases, care should be taken that the product from such manipulations is accepted by the consumers.

Biofortification as a strategy combines the use of both conventional and sometimes transgenic approaches for accumulation of carotenoids in storage parts (Bouis and Welch, 2010). In biofortification, the breeders aim at

Table 1. Major staple food crops in Africa, that have been biofortified for increased provitamin A content and their distribution.

Crop	Sweet potato (OFSPs)	Cassava (Yellow cassava)	Maize (orange maize)	Banana (Golden banana)
Varieties released	NASPOT 12 O, NASPOT 13 O, Kakamega (SPK004) Ejumula, NASPOT 9 O, NASPOT 10 O, NASPOT 7, NASPOT 8 Dimbuka- Bukulula, Resisto, Persistente, Tiba, LO-323	I011661 in DRC UMUCASS 36, UMUCASS 37, UMUCASS 38, TMS07/0593, TMS07/0539 TMS07/0220	MeruVAH517 MeruVAH519	Local varieties in DRC and Burundi, having appreciable levels are available
Level of pVAC (wild types)	30-100 ppm	0-19 ppm	0-19 ppm	1.4-11.3 ppm
Target level (pVAC)	32 ppm	15 ppm	15 ppm	Up to 20 ppm
Other variety characteristics	Yield = 60t/ha on station and 12t/ha farmer field, DMC (30-35%), moderate resistance to disease and pests	Yield, early maturity, tolerance to pests and diseases, DMC, pound-ability, mealiness, sweetness, ease of peeling, marketability, and in-ground storage	higher zinc content, competitive grain yield and consumer preferred end-use quality traits	No specific carotene rich varieties released so far
Country of release	Uganda, Mozambique. High adoption in all countries in SSA	Nigeria, DRC, Kenya, Ghana, Sierra Leone, Malawi	DRC, Ghana, Malawi, Mali, Nigeria, Rwanda, Tanzania, Zambia, and Zimbabwe	Trials in Australia and in East Africa
Target countries	East and Southern Africa	West Africa, East Africa and part of south Africa	Sub Saharan Africa and South Africa	East Africa, DRC
References	Mwanga et al. (2007), Mwanga et al. (2016), Laurie (2001)	Esuma (2016), Ssemakula and Dixon (2007), Ssemakula et al. (2008), Njoku et al. (2014)	Menkir and Maziya-Dixon (2004), Pixley et al. (2013), Ortiz et al. (2016)	Ekesa and Nabuuma (2016), Paul et al. (2017), Fungo and Pillay (2011), Mbabazi (2015)

providing mechanisms for accumulation of carotenoids in addition to reduction of ant-nutrient substances that inhibit carotenoid bioavailability after consumption. Relatedly, breeders can increase particular substances in the crop that stimulate and promote carotenoid bioavailability in storage parts or the crop product. If this is not done, then the breeding process would not yield products that are of use to consumers. This calls for a thorough appraisal of the agronomic performance of the crop which should be enhanced to allow for sustained crop performance (Welch, 2002).

As a strategy, transgenic approaches are important in crops where the carotenoids are absent or do not occur in such significant amounts as would be increased through conventional means (Bouis and Saltzman, 2017). This approach is precise in delivering significant amounts of nutrients to the crop in question and tends to shorten the breeding cycle. In addition to increasing carotenoid contents, the approach also ensures that specific agronomic and performance properties of the crop (especially sink size and activity) are maintained. However, the approach is affected by the highly risk averse regulatory approval processes as has been seen in the case of “Golden rice” (Wesseler and Zilberman, 2014).

Successful biofortification strategists like Harvest Plus have used specific approaches based on conventional breeding approaches. Such approaches are indeed faster and better means of getting the carotenoid rich crops to consumers.

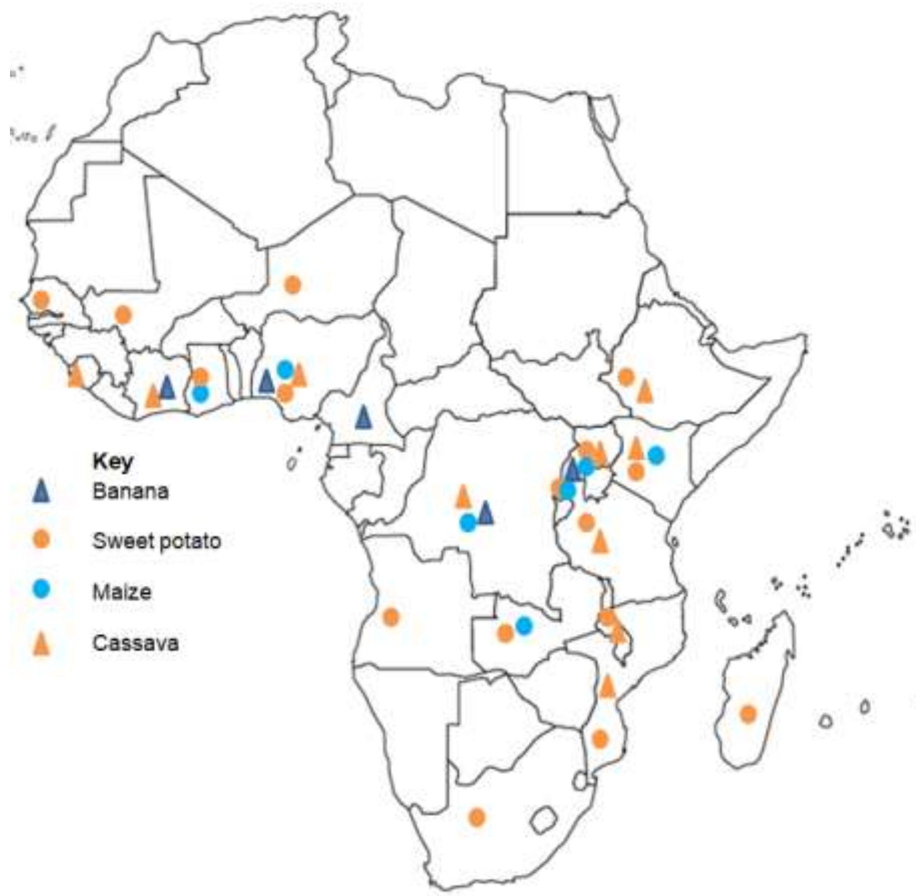

Figure 2. Map showing major staple food crops under biofortification for provitamin A enhancement in Africa and the countries involved in biofortification by 2017.
Source: Adapted and modified from HarvestPlus (2014).

The approaches involve the identification of varieties that are already adapted to a particular location of interest but also carry "significant carotenoid content". Such varieties are then packaged for release and/or dissemination as "fast track" varieties. This provides spot on solutions to populations in need and can complement the long and laborious carotenoid conventional breeding processes. In addition, harvest plus conducts a range of multi-locational trials across specific similar geographical locations that allow for testing and faster release of developed nutrient rich varieties (Bouis and Saltzman, 2017).

Therefore, it is important to note that conventional breeding can be coupled to modern genetic approaches for enhanced carotenoid increments in storage parts of staple crops. However, the application of genetic engineering approaches in delivering such products is still elusive. Thus the breeding of African staples for enhanced carotenoid concentrations has been based on conventional approaches. Such approaches have delivered important crops such as sweet potato, cassava, and maize that contain higher levels of provitamin A carotenoids (pVACs) (Table 1). They have also been used to deliver other micronutrient rich crops such as beans and a number of cereals.

Vitamin A biofortified crops for consumers in Africa

Sweet potato

Sweet potato is widely consumed in sub-Saharan Africa and was the first biofortified crop developed and released by the International Potato Center (CIP), HarvestPlus and their partners. It accumulates provitamin A up to 100 ppm exceeding the target level of 32 ppm (Andersson et al., 2017). The primary evidence for the effectiveness of biofortification in accumulation of carotenoids in storage parts comes from Orange Sweet Potato (OSP). In Uganda, orange-fleshed landrace cultivars named 'Ejumula' and 'SPK004' (Kakamega) (Mwanga et al., 2007), and developed varieties (NASPOT 9 O' (NASPOT 10 O, NASPOT 12 O and NASPOT 13 O) with yellow roots (sign of pVACs accumulation), were released in 2004, 2007 and 2013 (Mwanga et al., 2016). Biofortified

OSP varieties have been released in more than 15 countries across sub-Saharan Africa with a record adoption rates greater than 60% above control communities (Bouis and Saltzman, 2017). Introduction of these varieties resulted in increased vitamin A intakes among children and women, improved vitamin A status among children and decreased the prevalence of low serum retinol by 9% points (Mwanga et al., 2016). Women who consumed OSP also had a lower likelihood of having marginal vitamin A deficiency. Recent research on the health benefits of biofortified OSP in Mozambique showed that biofortification improved child health as indicated by reduced prevalence and duration of diarrhoea in children under five years of age (Bouis and Saltzman, 2017).

Cassava

Cassava is a dietary staple in much of tropical Africa, and grows well in poor soils with limited labour requirements. Total carotenoid concentration in fresh yellow cassava is primarily in the form of all-trans-β-carotene and is located in the parenchyma cells, the storage cells of the roots (Talsma, 2014). Breeding programmes for provitamin A cassava such as the International Center for Tropical Agriculture (CIAT) and the International Institute of Tropical Agriculture (IITA) generate high-provitamin A sources via rapid cycling in pre-breeding and provides in-vitro clones and seed populations for local adaptive breeding. Indeed, the breeding efforts at CIAT have already led to the generation of cassava genetic stocks that have accumulated up to 25 μg/g of β-carotene in fresh roots (Ceballos et al., 2013). In Nigeria, three first-wave provitamin A cassava varieties with 6–8 ppm of provitamin A (about 50% of the target) were released in 2011 followed by three other varieties with up to 10 ppm (66% of the target) in storage roots released in 2014. However the breeding target is to deliver varieties with up to 15 μg/g fresh weight of carotenoids in fresh roots (Talsma, 2014). National programmes have also released yellow cassava varieties in Ghana, Malawi, and Sierra Leone, and regional trials are underway for fast-tracking release in other countries in West and East Africa that have similar agro-ecologies. In Uganda, elite provitamin A cassava germplasm was introduced from CIAT and IITA around 2012 (Esuma et al., 2012) for evaluation under local field conditions. Breeding efforts have currently given rise to varieties with carotenoids content up to 12 μg/g, awaiting release for farmer adoption (Esuma et al., 2012). As earlier stated, this is affected by the negative correlation between carotenoid increments and dry matter accumulation hence the need to combine high carotenoid content and high dry matter content of biofortified germplasm for Africa. As earlier stated, adoption of provitamin A varieties by farmers is hindered by the negative correlation between carotenoid content and dry matter content, hence the need to improve the dry matter content of biofortified germplasm for Africa.

Maize

Maize is the most important cereal crop in sub-Saharan Africa and is also an important staple in Latin America. Initial screening of more than 1,500 maize germplasm accessions found ranges of 0–19 ppm provitamin A in existing maize varieties, exceeding the provitamin A target of 15 ppm (Menkir et al., 2014; Andersson et al., 2017). Provitamin A maize breeding programs at the International Maize and Wheat Improvement Center (CIMMYT), IITA, and the Zambia Agriculture Research Institute (ZARI) began in 2007. Both hybrid and open-pollinated (synthetic) biofortified varieties are being developed for with improved carotenoid storage in the grain (Andersson et al., 2017). In Africa, more than 40 provitamin A maize synthetic hybrids, single-cross hybrids, and three-way hybrids have been released in the DRC, Ghana, Malawi, Mali, Nigeria, Rwanda, Tanzania, Zambia, and Zimbabwe (Andersson et al., 2017). The first wave of varieties released in 2012/2013 contained 6-8 ppm additional provitamin A (about 50% of the target increment) in the dry grain, while second-wave varieties (released in 2015/2016) contained about 10 ppm additional provitamin A (66% of the target increment). Varieties that fully meet the provitamin A target level are being tested in multi-location trials across sub-Saharan Africa and are expected to be released in 2018 (Andersson et al., 2017). All biofortified varieties combine competitive grain yield and consumer preferred end-use quality traits with higher provitamin A content.

Banana

Banana is an important staple food and source of income for over 100 million people in Sub-Saharan Africa, with consumption averaging 300 kg per person per year in the East African highlands and the Great Lakes region of Africa (UNSCT, 2007). The high consumption rate makes banana an important source of carbohydrates, vitamins and minerals in the diets of these populations (Davey et al., 2007). However, most of local cultivars have significantly lower levels of pro-vitamin A in the fruit and are consumed in a region where VAD deficiencies range from 39-50% (IFPRI, 2016) and way beyond the WHO acceptable intervention level of 15% (WHO, 2009). Thus the inherent potential of these cultivars for improvement into pVAC-rich cultivars with organoleptic properties that compare well with that of local cultivars must be harnessed.

Evaluation of some of the banana genotypes have already shown a wide variation in provitamin A carotenoids (pVACs) content with values as high as 220 nmol g^{-1} dry weight (DW) (Davey et al., 2009). Other studies by Ekesa et al. (2013) in popular banana cultivars in Eastern Africa reported pVAC ranges from 7 to 27 nmol g^{-1} DW. These can be improved using germplasm from other sources that have higher levels of pVACs than local cultivars (Ekesa et al., 2013; Englberger et al., 2003; Fungo and Pillay, 2011). On the basis of consumer reliance on banana for food and the high bio-accessibility of vitamin A in banana (Ekesa et al., 2013), pVAC-rich banana cultivars form a vital route that answers to the high levels of prevalence of VAD within Eastern Africa.

Notwithstanding efforts for biofortification of banana with provitamin A carotenoids in Africa, the most outstanding successes are still on the proof of concept. PVA-biofortified transgenic Cavendish bananas were generated in collaboration with African partners and field trialed in Australia with the aim of achieving a target level of 20 lg/g of dry weight (DW) b-carotene equivalent (b-CE) in the fruit (Paul et al., 2017). However, these have not been deployed in the greater banana region of east Africa. Needless to say, it is evident that a shift from low-carotenoid to high-carotenoid banana cultivars would lead to increased vitamin A content of the diet and thus possibly lead to improved vitamin A status among consumers.

RETENTION OF CAROTENOIDS AND VITAMIN A DURING FOOD PROCESSING

Staple crop storage parts processing results into reduction in carotenoids content. The reduction in carotenoids content during processing differs: 1) from variety to variety (Chavez et al., 2007; Vimala et al., 2011); 2) from one processing method to the other (Vimala et al., 2011; Chavez et al., 2007) and 3) from different positions of the same storage part within the variety (Talsma, 2014). Talsma (2014) attributed the variations in carotenoids reduction among different positions within the same variety to the variable distribution of dry weight matter within a particular storage part. Carotenoids retention in staple crops products may vary from as low as 10% for heavily processed and roasted food granules, to about 87-90% in less processed storage parts (Ceballos et al., 2012; De Moura et al., 2015).

Generally, increased temperature and light conditions severely reduce the amount of available carotenoids. Reduced retention is mainly through a number of degradative pathways including the reaction of carotenoids with atmospheric oxygen (autooxidation), light (photodegradation) and heat (thermal degradation). Degradation can also be as a result of interactions of carotenoids with singlet oxygen, acid, metals, and free radicals within the product processing environment.

Given the complex nature of food based material matrix from biofortified crops, the retention/degradation of carotenoids is a complex process that is as a result of a range of factors. Carotenoid interaction with other materials/biomolecules within the food is rather not well understood (De Moura et al., 2015). However, various studies have focused on the understanding of carotenoid retention on the effect of external factors/processes on carotenoid availability. Thus it remains to be understood, on the exact physiological processes that take part in the degradation of carotenoids and hence reducing carotenoid retention.

From the review of different studies undertaken on a range of biofortified crops deployed especially in sub Saharan Africa (De Moura et al., 2015), retention levels have been elucidated and henceforth, recommendations on the same can be made (Figure 3). It was shown that in sweet potato, carotenoid retention is high if the food matrix is processed by boiling and/or production of porridge. Such processes retain carotenoid content well above 90% and would be ideal for the African settings. In maize, the best method of processing was making of porridge (80% retention) while in cassava, boiling and *fufu* production were scored as best method for high retention (over 90%). Among such boiling procedures as the most common form of food preparation, boiling with minimal water (half full) resulted into higher retention for carotenoids. Most of the biofortified crops in Africa are primarily processed by drying. Among the drying procedures, it was realised that shade and solar drying resulted in lesser degradation and hence higher carotenoid retention. Since most farmers store their produce after harvest, the recommended storage procedure among different crops that retains higher levels of carotenoids was storage in jute bags. It was also realised that the storage of the food material in the dark gave better results. Such information is critical in deployment of biofortified crops if at all, their intended benefits are to be realised by the intended beneficiaries.

CONCLUSIONS

Like other plants, African staple crops accumulate carotenoids through specified biosynthetic processes. However, for most crops that have consumer-accepted attributes, the level of carotenoid accumulation is low and may not cater for the nutritional requirements of the consumers. Through biofortification, staple crop carotenoid contents can be significantly improved as has been demonstrated in sweet potato, cassava, bananas and maize. Such improvements take into account the apparent variability in crop carotenoid contents and hence varietal improvements would best target varieties or cultivars that already contain appreciable amounts of carotenoids. On the other hand, such varieties may not carry specific consumer preferred traits. Hence, African breeding programs have created breeding pipelines for

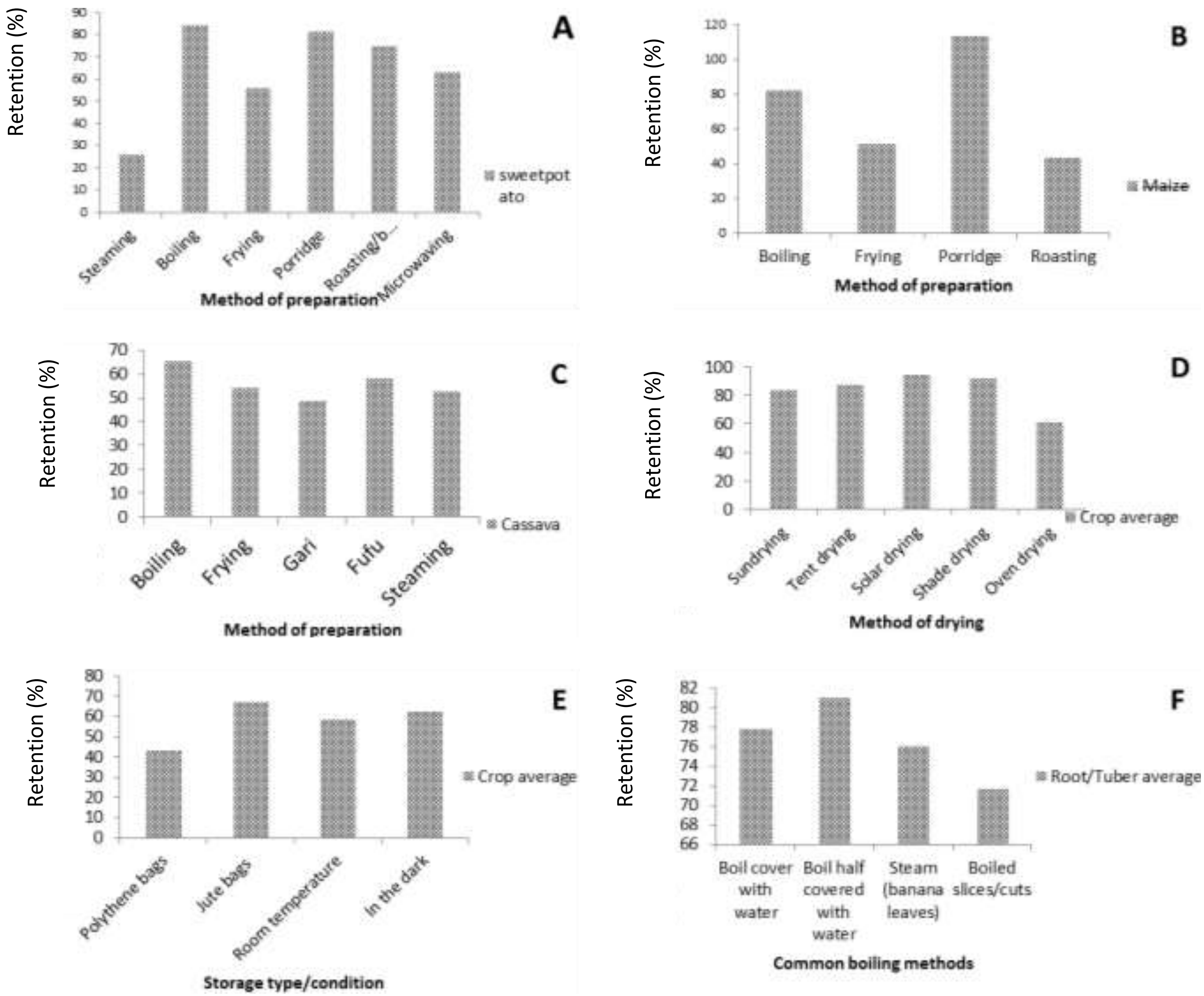

Figure 3. Retention of carotenoids in crops under different conditions. A= Average Carotenoid retention in Sweet potato; B=Average Carotenoid retention in Maize; C=Average carotenoid retention in cassava D=Crop average for carotenoid retention in various drying methods; E=Crop average for carotenoid retention during storage in bags or different light conditions; F=Root/Tuber average carotenoid retention during boiling.
Source: Adapted from De Moura et al. (2015).

the production of vitamin A rich crops.

These breeding pipelines have produced a range of varieties which have been adopted by farmers in different parts of Africa. The efforts have been very successful especially in sub Saharan Africa with almost all the countries having biofortified crop varieties. However, challenges still remain such as accumulation of the right amounts of carotenoids in storage parts to allow bio-accessibility, acceptability of the crop or their products, the susceptibility of such crops to diseases and pests and the retention of such carotenoids within the processed products meant for consumption.

Such challenges indicate the need for concerted efforts in breeding, food science, post-harvest technology and other in providing for highly bio-accessible, highly retainable and useful forms of these nutrients in staple crops. This would take the form of the clear understanding of the molecular and physiological aspects of carotenoid accumulation in crops plants. It would also require specific solutions related to appropriate processing procedures for these crops coupled with an understanding of the interaction of carotenoids with other molecules in the food matrix. Solutions to a range of challenges related to utilisation of biofortified would henceforth result into their increased utilization and possibly reduce the rampant micronutrient related disorders in Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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