A color chart to screen for high β-carotene in OFSP breeding

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Abstract

Orange fleshed sweetpotato (OFSP) varieties are considered as the first biofortified varieties among major food crops. About 100g fresh sweetpotato storage roots or less contain enough β-carotene to provide the daily pro-vitamin A needs of a pre-schooler. However, determination of β-carotene by chemicals methods is expensive. Since the storage root flesh color is highly correlated with the β–carotene concentrations in sweetpotato, color charts can be used to breed β–carotene rich sweetpotato varieties with nearly zero costs. The objectives of this study were to determine the β–carotene concentration of sweetpotato storage roots with a wide range of colors, to characterize the flesh color by color charts, and to associate each color with the corresponding β–carotene concentration. A total of 248 roots coming from 31 genotypes (2 roots per plot, 2 plot replications and 2 environments: La Molina and San Ramon) were classified by their flesh color using the Royal Horticulture Society color chart. Freeze dried samples of every roots were prepared and analyzed for total and β-carotene concentration by HPLC. Most of the roots showed a first and a secondary flesh color. Roots were grouped in 10 groups according to its primary flesh color. All roots with deep orange and orange primary flesh color showed significant β–carotene concentration (above 4 mg / 100 g fresh weight or 300 ug RAE). The β–carotene concentration of intermediate and pale orange primary flesh color ranged from 0.5 to 8 mg / 100 g fresh weight depending on the color intensity of the primary or secondary colors and their proportion. Roots with yellow orange, pale orange, yellow, intermediate yellow, pale yellow and cream primary flesh color had only very low amounts of β-carotene. The evaluated color chart might be a useful tool in breeding programs to select for high β–carotene cultivars.

Keywords: sweetpotato, β–carotene, color chart, breeding.

Introduction

The potential of orange-fleshed sweetpotato to contribute to a food-based approach to combating VAD in Sub-Saharan Africa has been shown (Hagenimana and Low, 2000; Low et al., 2001). About 100g of orange-fleshed sweetpotato have a β-carotene content of 60 µg/g on fresh matter basis and can provide all the Recommended Daily Intake (RDI) of vitamin A for children (450 ug RAE/day, FAO/WHO, 2002). The efficacy of β-carotene-rich orange-fleshed sweetpotato variety Resisto in improving the vitamin A status has recently been demonstrated in South African primary school children (van Jaarsveld et al., 2005). However, orange-flesh is associated with low dry matter and the preference in Sub-Saharan Africa is a high dry matter sweetpotato, which is usually white or cream flesh and low in β-carotene concentration.

Sweetpotato breeding at International Potato Center (CIP) is ongoing to increase the dry matter content of β-carotene-rich orange fleshed sweetpotato and to improve the sensory characteristics, and at the same time to increase resistance to viruses and stress. Since often thousands of genotypes need to be evaluated in breeding, the screening for high β–carotene genotypes requires simple, fast and inexpensive methods. A strategy to select for high β–carotene genotypes is to screen the storage root color first - using a color chart - and eliminating genotypes with faint color; then to screen by NIRS to eliminate more genotypes and finally to select few genotypes which are submitted to the costly HPLC analysis. Official descriptors for sweetpotato include the use of a color chart for flesh color characterization (CIP/AVRDC/IBPGR, 1991), however this color chart does not include the ample range of yellow and orange flesh colors that sweetpotato flesh can have. Additionally, there is not a clear definition of which colors are related with a significant pro-vitamin A concentrations. Furthermore, the root flesh color is mostly a combination of colors which makes more difficult the color classification

The objectives of this study were to determine the β–carotene concentration of sweetpotato roots with a wide range of colors, characterize the flesh color, and to associate each color with the corresponding β–carotene concentration in order to generate recommendations for the use of a color chart for selecting for high β–carotene sweetpotato clones in a breeding program.

Materials and methods

Plant material, sampling and sample preparation

Sweetpotato roots samples were taken from 31 genotypes, which were grown in La Molina and San Ramon, Peru. For each genotype, 2 roots per plot in the field were gathered at random and brought to the laboratory. Each root was washed with tap water, peeled, longitudinally halved, classified according to flesh color and then quartered. Three to four slices of two opposite wedges were taken to comprise a fresh root sample of 50 g approximately. The fresh sample was placed in a plastic bag, frozen at –20°C, dried in a freeze dryer, milled, placed in a polyethylene bag and stored at -20°C until carotenoid analysis. In total 248 samples were prepared.

Flesh color classification

Halved storage roots were observed and each root was classified according its primary and secondary flesh color using the Royal Horticulture Society color chart. The primary flesh color was defined as the font or principal color in the flesh and the secondary color was defined as the spots and veins colors in the flesh. The colors described in the RHS color chart were grouped in 10 groups, as follows:

Group 1: Deep orange-fleshed clones. Primary flesh color similar to 28A, 28B, 30C and 30D (dark tangerine orange)

Group 2: Orange-fleshed clones. Primary flesh color similar to 24A, 25A, 25B, 25C, 26A and 26B.

Group 3: Intermediate orange-fleshed clones. Primary flesh color similar to 24C, 26C, 26D, 28C, 28D, 29A and 29B.

Group 4: Pale orange-fleshed clones. Primary flesh color similar to 23D, 24D, 25D, 27A, 27B, 27D, 29C and 29D.

Group 5: Yellow orange-fleshed clones. Primary flesh color similar to 15D, 18A, 20B, 16B and 16C.

Group 6: Pale yellow-orange-fleshed clones. Primary flesh color similar to 14D, 15D, 16D, 18B, 18C, 18D, 19B and 20C.

Group 7: Yellow fleshed clones. Primary flesh color similar to 2C, 4B, 5C, 6C, 7D, 8A, 8B, and 13C.

Group 8: Intermediate yellow fleshed clones. Primary flesh color similar to 10A, 10B, 10C, 11B and 12C.

Group 9: Pale yellow fleshed clones. Primary flesh color similar to 2D, 8C, 9D 10D and 11C.

Group 10: Cream fleshed clones. Primary flesh color similar to 8D, 11D, 12D and 13D.

Total and individual carotenoid determinations

Carotenoid analysis was carried out according to Kimura et al. (2007). Briefly, 0.1 - 1g of the freeze dried and milled sweetpotato sample was extracted with acetone by grinding with a mortal and pestle. Extraction was repeated until the residue was devoid of color. The resulting extracts were brought to a volume of 25ml with petroleum ether. The total carotenoid concentration was calculated using the absorbance value measured in a spectrophotometer (Shimadzu UV 160A) at 450nm and the extinction coefficient for mixtures of β-carotene (2592) (Davies, 1976). For individual carotenoid analysis, 15 mL of the extract were dried with nitrogen gas (N2), redissolved in 1 mL of HPLC grade acetone (Fisher) and filtered through a 0.22 µm PTFE syringe filter (Millipore). In total 10 µL of the filtered extract were injected into a Waters HPLC machine, equipped with a separation system (model 2995), quaternary pump, autosampler, in line degasser and photodiode array detector (model 2696) controlled by Empower software. Separation was carried out on a YMC C30 polymeric column (3µm, 4.6 x 250mm) using as mobile phase an isocratic elution of methanol:methyl-tert-butyl eter (80:20) with a flow rate set as 0.8 ml/min. Detection of β-carotene was done at maximum absorption wavelengths of 450nm. Identification of the β-carotene was based on the combined analysis of the retention times, co-chromatography with pure

standards from Sigma and CaroteNature (Lupsingen, Switzerland) and the visible absorption spectra obtained by the photodiodide array detector. Quantification was done by external calibration. Total carotenoids and β-carotene were expressed as micrograms (µg) per 100 g fresh weight (FW).

Results and discussion

The range of variation for the β-carotene and the total carotenoid concentrations in each of the 10 groups formed according to the primary flesh color is given in Table1.

Table 1. The β-carotene and the total carotenoid concentrations in 10 groups formed according to the primary flesh color

Group	N	mg / 100g, FW		ug RAE / 100 g, FW	
		β-carotene range	Total carotenoid range	A[†]	B[‡]
Deep orange	50	4.29 - 18.55	6.46 - 24.26	357 - 1546	253 - 1082
Orange	7	5.08 - 6.12	7.51 - 10.59	424 - 628	351 - 434
Intermediate orange	30	2.08 - 8.36	4.09 - 11.73	173 -696	121 - 487
Pale orange	15	0.56 - 4.47	0.73 - 8.32	47 – 372	32 - 260
Yellow orange	12	0.16 - 2.60	1.03 - 5.06	14 – 216	10 - 152
Pale yellow orange	48	0.02 - 2.51	0.68 - 5.60	1.5 – 208	1.1 - 146
Yellow	14	0 - 0.28	0.76 - 2.98	0 – 23	0 - 16
Intermediate yellow	20	0 - 1.32	0.86 - 3.19	0 – 110	0 - 77
Pale yellow	27	0 - 1.47	0.39 - 4.40	0 – 123	0 - 86
Cream	25	0 - 0.48	0.00 - 4.43	0 - 40	0 – 28

[†]A: RAE considering 12 ug of β-carotene to be equivalent to 1 ug of retinal (IOM, 2001)

[‡]B: RAE considering 12 ug of β-carotene to be equivalent to 1 ug of retinal (IOM, 2001) and assuming a 70% retention after boiling

Roots with orange and deep orange as primary flesh colors showed concentrations of β-carotene and total carotenoids ranging from 4.29 to 18.55 and 6.46 to 24.26 mg / 100g FW, respectively. The β-carotene range is similar to that found for other orange-fleshed varieties (6.7 - 128 mg / 100g FW) by Huang et al. (1999) and by van Jaarsveld et al. (2004) for the variety Resisto (13.2 to 19.4 mg / 100g FW). Storage roots with intermediate orange primary flesh color showed concentrations of β-carotene and total carotenoid concentrations ranging from 2.08 to 8.36 and 4.09 to 11.73 mg / 100g FW, respectively. Considering 12 ug of β-carotene to be equivalent to 1 ug of retinal (IOM, 2001) and assuming a 70% retention after boiling, 100g of the orange and deep orange roots evaluated in this study provide between 56 and 241% of the RDI of vitamin A for children under five years old (450 ug RE/ day; FAO/WHO, 2002) and 100g of the intermediate orange roots provide between 27 and 108% of this recommendation.

Most of the orange or deep and intermediate orange fleshed roots showed a secondary color storage root (pale yellow orange, pale orange and intermediate orange). The wide range of variation in carotenoid concentrations could be explained by two factors: (i) the color intensity of the primary or secondary colors and (ii) the proportion of the secondary color with respect to the primary flesh color. For example, two storage roots with the same primary (deep orange) and secondary (intermediate orange) flesh colors (Fig. 1 a and b). However, the fact that the secondary color represents a bigger proportion with respect to the primary color in the first storage root compared to the second storage root results in higher β-carotene concentrations in the second storage root (12.39 mg / 100 g, FW) compared to the first storage root (7.76 mg / 100 g, FW). Another example is shown in Fig. 1c and d. Both storage roots are intermediate orange for primary and secondary flesh color. However the fact that the secondary color represent a bigger proportion with respect to the primary color in the first storage root

compared to the second storage root results in higher β-carotene concentration in the second storage root compared (7.23 mg / 100g, FW) compared to the first storage root (4.61 mg / 100g, FW).

Roots with pale orange as the primary flesh color showed β-carotene and total carotenoid concentration ranging from 0.56 to 4.47 mg / 100g FW, and from 0.73 to 8.32 mg / 100g FW, respectively. Pale orange fleshed roots showed high β-carotene concentration when the secondary color were orange or intermediate orange and when the secondary storage root color had a large proportion (some small spots or veins) of the flesh (above 3.00 mg / 100g FW) (Fig. 2). Roots with pale yellow orange, yellow, intermediate yellow, yellow orange, cream and pale yellow showed very low β-carotene concentrations (Table 1).

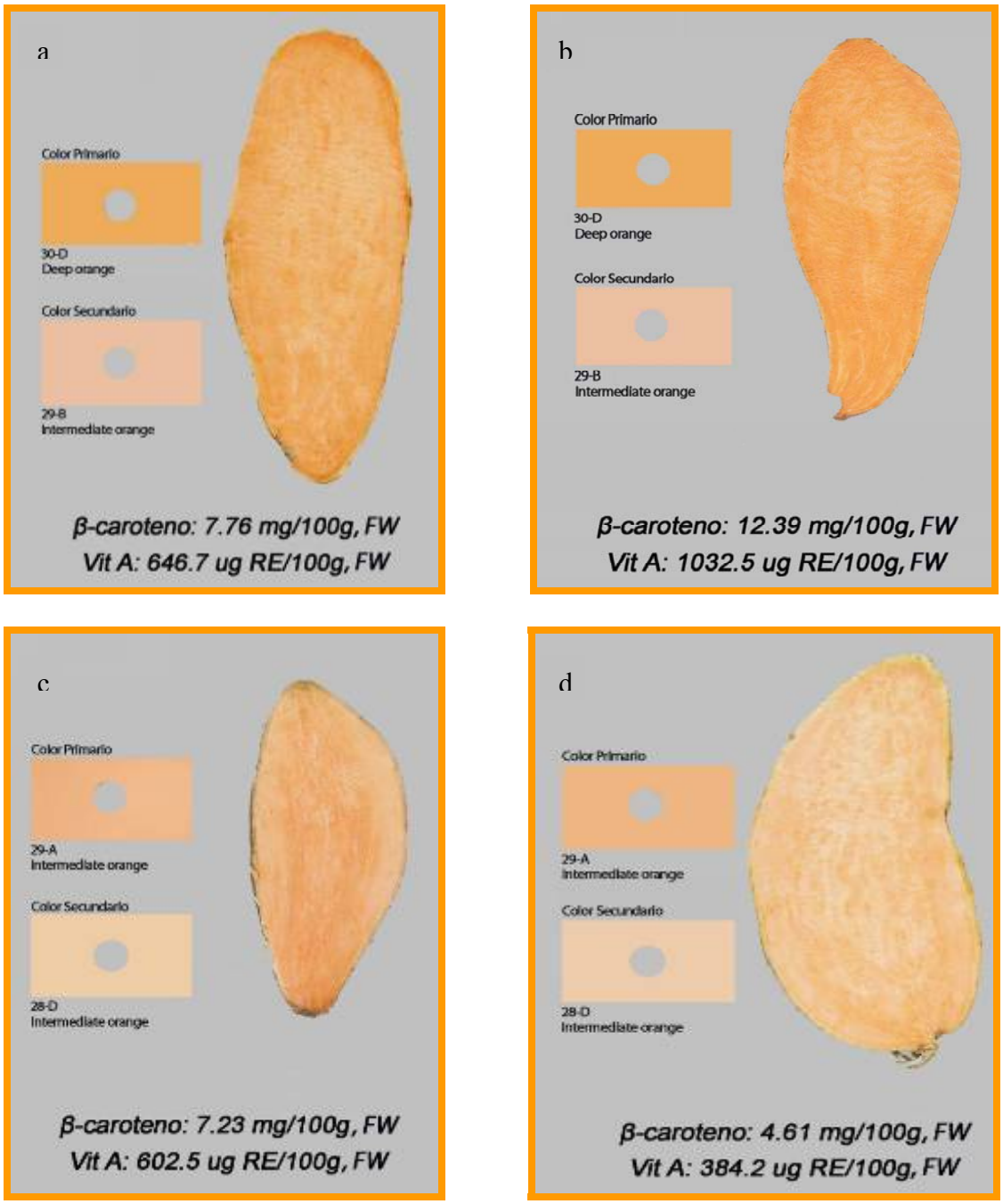

Figure 1. Examples of sweetpotato storage roots used in the developed color chart.

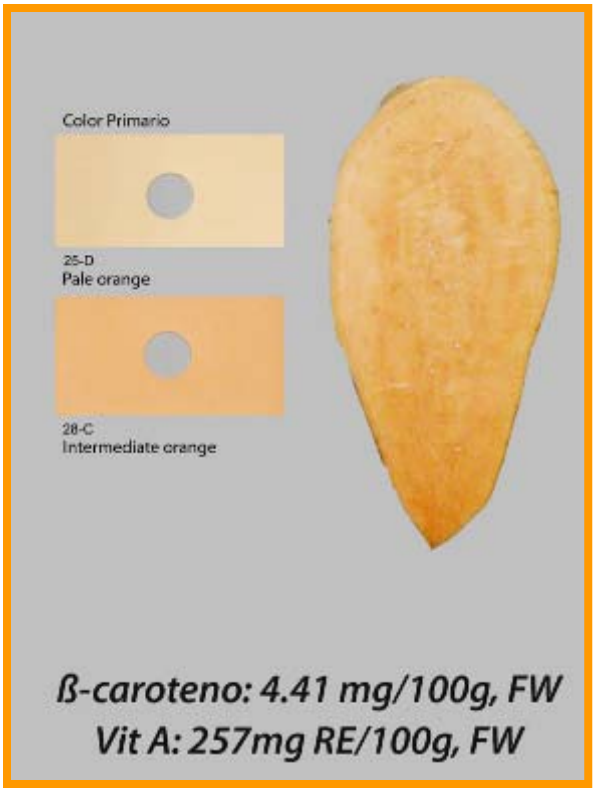

Figure 2. Roots with pale orange as the primary flesh color

Recommendations

Assuming a 70% retention after boiling, 100 g of roots with 3 mg of β– carotene would provide more than 35% of the RDI of vitamin A for children under five years (450 ug RE/ day; FAO/WHO, 2002). Taking this into account, recommendations for selecting high β-carotene roots by using the RHS color chart are::

3. Roots with orange, deep orange and intermediate orange as primary flesh color and orange and intermediate orange as secondary colors have a significant amount of β-carotene.
4. Roots with intermediate orange as primary flesh color and pale orange as secondary colors have a significant amounts of β-carotene only if the secondary color represents only small proportion of the primary color.
5. Roots with pale orange as primary flesh color have a significant amount of β-carotene only if the secondary colors are orange and intermediate orange and represent a big proportion of the primary color.
6. Roots with yellow orange, pale orange, yellow, intermediate yellow, pale yellow and cream have no significant amount of β-carotene.

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