

Potentiality of Cassava Cultivars as a Source of Carotenoids

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1 . Abstract

Vitamin A deficiency results in progressive eye damage. It is a serious problem in the northern and northern east Brazil. Screening some cassava clones and interspecific hybrids for its precursors revealed high level of lutein and trans-B-carotene in roots of the indigenous Brazilian clone namely UnB 400 which is popularly known by (Amarela). It has its roots content reaching 236 and 1.24 mg/g respectively combined to an excellent palatability . An interspecific hybrid of cassava with *M. oligantha* showed its leaves to have 9108 mg/kg compared to 780 mg/kg lutein in common cultivars.

Key words: Carotene, Protein content, interspecific hybrid, palatability

Introduction

Vitamin A deficiency results in progressive eye damage. It is a serious problem in the northern and northern east Brazil(1,2,3) and many other parts of the country (4,5,6,7).

Vitamin A deficiency ranges from night blindness to those of xerophthalmia and keratomalacia, leading to total blindness. Furthermore, it exacerbates afflictions such as diarrhea, respiratory diseases, and childhood diseases such as measles (8,9). The principal vitamin A source is from animal origin, this has an elevated cost to marjory of Brazilian population. The pro-vitamin A carotenoids are cheaper source since they are found abundantly in plants.

Carotenoids are a auxiliary photosynthesis pigment. Some of them are converted in vitamin A (e.g. trans-b-carotene). They have other functions besides the provitamin A activity, such as heart disease and cancer prevention, lower risk of cataract and macular disorders, and immunoenhancement (10, 11). In these cases the carotenoids act as an antioxidant agent.

Cassava is one of the most important sources food in several tropical countries and regions in the world particularly Northern and Northern east Brazil. It is estimated that it sustains more than 800 millions people (12). Selecting high carotene content clones of it may contribute significantly to resolve problem of Vitamin A deficiency in poor countries. This will help too in ability of this crop to cope with unfavorable environmental conditions.

The average requirement of B carotene recommended by WHO is 2.4 mg up to 3.5 mg for adults. We try here to evaluate the range of content of the carotene in cassava and determine how much possibility select of clones rich in carotene combined to good palatability.

Materials and methods

Extraction

10 grams of mature roots and 5 grams of leaves were extracted three times with acetone (5ml per gram). The filtered acetone extract was added in separation funnel containing petroleum ether, distilled water, ethylic ether (100,100,0.3: v,v,v). The aqueous fraction were discarded, and the organic fraction were submitted to saponification.

Saponification was preferred since it removes accompanying lipids and chlorophylls. In our work, the optimal conditions for mild saponification achieved with 10 % Methanolic potassium hydroxide solution (100ml) overnight at room temperature.

After saponification the aqueous fraction were discarded and the organic fraction were dried with sodium sulphate anhydrous. The organic fraction were concentrated to 1 ml and submitted to HPLC system.

Equipment

Carotenoid analyses were performed by Shimadzu LC-10A HPLC equipped with a photodiode array detector SPD MXA-10 and a Rheodyne injection valve with 20 mL loop. The separation was carried out on a C18 Vydac 218TP54 column 250x4.6 mm i.d. (5 mm particle size), with 100% MeOH as mobile phase at a flow rate of 1 mL/min at temperature of 15 °C. The chromatograms were processed at wavelengths of maximum absorption (450 nm). The carotenoids identification were achieved by retention time (tR) comparisons with those of the standard compounds and using the wavelength of maximal absorption (Imax) and the shape of the spectrum between 300-600 nm compared with data available in the literature (13,14).

Quantification

The calibration curves for trans-b-carotene (Purchased from Sigma Inc.), a-carotene and lutein (Purified from alfafa) were constructed with a minimum of the concentration levels, each in triplicate. All curves showed a good relation of area and concentration achieving R2 of 1.00, 0.98 and 0.99, for trans-b-carotene, a-carotene and lutein respectively. The cis isomer of b-carotene were quantified using the calibration curve of trans-b-carotene.

The vitamin A values were calculated according to the conversion factor given by NAS-NRC (15) whereas 6 mg of trans-b-carotene correspond to 1 mg of retinol equivalent (RE), and the activities are related as follows: 100% for trans-b-carotene, 50% and for trans-a-carotene and cis-b-carotene (16).

Results and Discussion

All the cassava clones presented the same major carotenoids in different concentrations. Figure 1 and 2 show the most common carotenoid pattern present in roots and leaves respectively. The identification and characterization of the peaks are presented in Table 1.

Lutein (peak 3), trans-a-carotene (peak 6) and trans-b-carotene (peak 7) coeluted with their specific standards, showing UV-visible spectra similar to those presented by Rodriguez-Amaya (17). Separation of 9-cis-b-carotene (peak 9) and 13-cis-b-carotene (peak 10) was not achieved.

The identification of Violaxanthin, 5,8-Epoxy-lutein, b-Cryptoxanthin, cis-b-Cryptoxanthin, Phytofluene was achieved using the Imax peaks (14) and the shape of the spectrum between 300-600 nm. (13, 14).

The colorimetric method for cassava clones characterization in terms of paranchymal color proved useful and pragmatically used to detect the variation exists. Among the cassava clones studied, the most impressing one is the indigenous Brazilian UnB-400 known popularly by Amarela having 236 mg/kg of Lutein compared to zero in other cultivars. This antioxidant material is extremely important for health conditions of poor people. The same clone has a reasonable quantity of 2.2 mg/g which is considered by WHO sufficient for daily requirements of adults considering the person consume normally a half kilogram of cassava daily. The presence of lutein with the immense quantity adds to

the valuable importance of this cultivar.

In tests of palatability of this clone it showed one of the most favoured one. It is easy cooked within 5 to 10 minutes maximum turning to a very soft mass like a cream. Very low in HCN content judging from its taste. In an ongoing trials a tetraploid line has been produced from this clone which candidate it to increase carotene content to more 50%. This clones an indigenous clone planted in the Federal district by varios farmers. It is known and produced also in different states of Brazil. There is reports it is planted also in Mosambique. However this is the first report on its high carotenoids content. Certainly it as been maintained by native people because of its excellent palatability and cooking quality. It shows how much valuable are indigenos genetic resources of a crop in its center of origin.

The most striking result is the content of both trans-b-carotene and lutein in leaves of clones UnB-400 and ICB-300. The trans-b-carotene reached 27.40 mg/g in the first clone making one of the most rich sources available for poor people for this precursor of Vitamin A, while ICB-300 has almost 20 mg/g of this type of carotene, making it a very rich source too. This clone; ICB-300 is the hybrid developed by the first author by hybridizing cassava with the wild relative *M. oliganth*. It has 5% protein compared to 1.5-2% in common cassava (18). The amazing result yet is the 3081 and 9108 mg of lutein in UnB-400 and ICB-300 respectively. These quantities means that they have about 4 to 12 times more than normal clones. Apparently the hybrid ICB 300 having 4% protein in the roots, 20 mg/g trans-b-carotene, 9108 mg/g of lutein in the leaves is an excellent source of these important components.

UnB-400 is a very good source of precursor of Vitamin A, considering its excellent palatability. UnB 300 is very good ingredient to be added to wheat flour forming bread considering its high protein and carotene content. Brazilian government is looking now to mix cassava flour with wheat to reduce importations of the latter one. An obstacle though is the low level of protein in common cassava compared to the 7% in wheat. Using the flour of the hybrid ICB 300 may resolve this problem. Being rich in vitamin A precursor in its leaves adds an advantage to its future use. These results refers to necessity of evaluating cassava interspecific

Hybrids for carotene content in the future since a previous study of the first author showed the cassava hybrid with *M. dichotoma* to have a double content of caroten i.e 22 mg/kg compared to 13 mg/kg in the common one (19).

Conclusion

Screening some selected cassava clones and its hybrid with a wild relative for carotene content, it was found that a Brazilian indigenous cassava clone namely UnB-400, popularly known by amarela has its roots rich in lutein and trans-b-carotene. It has an excellent palatability. The hybrid ICB 300 has a high caroten content in its leaves combined to high protein content in roots. It is rich too in lutein. The evaluation of both lutein and trans-b-carotene in leaves of the above two clones showed them immensely rich in trans-b-carotene having 20 mg/g and 27 mg/g respectively. For the lutein they have 9108 mg/g and 3081 mg/g respectively.

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References

1. Simmons, W. K. (1976). Xerophthalmia and blindness in Northeast Brazil. *Am. J. Clin. Nutr.* 29, 116-122.
2. Flores, H. and Araújo, C. R. C. (1984). Liver levels of retinol in unselected necropsy specimen: A

3. Dricot-d'Ans, C., Dricot, J. M., Diniz, A. S., Mariath, J. G. S., and Santos, L. M. P. (1988). Geographic distribution of xerophthalmia in the state of Paraíba, Northeast Brazil. *Ecol. Food Nutr.* 22, 131-138.
4. Desai, I. D., Tavares, M. L., Dutra-de-Oliveira, B. S., Douglas, A., Duarte, F. A. M., and Dutra-de-Oliveira, J. E. (1980). Food habits and nutritional status of agricultural migrant workers in Southern Brazil. *Am. J. Clin. Nutr.* 33, 702-714.
5. Wilson, D. and Nery, M. E. S. (1983). Hypovitaminosis A in Rio Grande do Sul, Brazil. Preliminary study. *Int. J. Vitam. Nutr. Res.* 24, 35-44.
6. Favaro, R. M. D., De Souza, N. V., Batistal, S. M., Ferriani, M. G. C., Desai, I. D., and Dutra-de-Oliveira, J. E. (1986). Vitamin A status of young children in Southern Brazil. *Am. J. Clin. Nutr.* 43, 852-858.
7. Gonçalves-Carvalho, C. M. R., Amaya-Farfan, J., Wilke, B. C., and Vencovsky, R. (1995). Prevalência de hipovitaminose A em crianças da periferia do município de Campinas, São Paulo, Brasil. *Cad. Saúde Públ.* 11, 85-96.
8. Grant, J. P. *The State of the World's Children* (Oxford Univ. Press, Oxford, 1991).
9. West Jr, K. P., Howard, G. R., Sommer, A., (1989). *Annu. Rev. Nutr.* 9, 63
10. Krinsky, N. I. (1994). The biological properties of carotenoids. *Pure Appl. Chem.* 66, 1003-1010.
11. Van den Berg, H., Faulks, R., Granado, H. F., Hirschberg, J., Olmedilla, B., Sandmann, G., Southon, S., & Stahl, W. (2000). The potential for the improvement of carotenoid levels in foods and the likely systemic effects. *Journal of the Science of Food and Agriculture*, 80, 880-912.
12. FAO 2003 Production Yearbook, Rome
13. Davies, B. H. (1976). Carotenoids. In: *Chemistry and Biochemistry of Plant Pigments* (T. W. Goodwin, Ed.), Vol. 2, pp. 38-165. Academic Press, London
14. Bauerfeind, J. C. (1972). Carotenoid vitamin A precursors and analogs in foods and feeds. *J. Agri. Food Chem.* 20, 456-473.
15. NAS-NRC (1989). *Recommended Dietary Allowances*, pp. 78-92. National Academy of Science, Washington.
16. Britton, G. (1995). UV/visible spectroscopy. In *Carotenoids*, Vol. 1B: Spectroscopy (G. Britton, S. Liaaen-Jensen, and H. Pfander, Eds.), pp. 13-62. Birkhäuser, Basel
17. Rodriguez-Amaya, D. B. (1999). *A guide to carotenoid analysis in food*. ILSI Press, USA.
18. Nassar, N. M. A. and Dorea, G. (1982). Protein contents of cassava cultivars and its hybrid with *Manihot* species. *Turrialba* 32: 429-432.
19. Nassar, N. M. A. ;Alves J. and de Souza, E. 2004. UnB 33: Na interesting interspecific cassava hybrid. *Revista Ceres* 51: 495-499.

Legend for Figures

Figure 1. Chromatogram profile of (A) Cassava root UnB-400 cultivar, Peak identification is given in Table 1. HPLC analysis conditions: RP column C18 Vydac 218TP54 column 250x4.6 mm, mobile phase 100% MeOH, flow 1.0 ml/min.

Figure 2. Chromatogram profile of (A) Cassava leaves from UnB-400 cultivar, Peak identification is given in Table 2. HPLC analysis conditions: RP column C18 Vydac 218TP54 column 250x4.6 mm, mobile phase 100% MeOH, flow 1.0 ml/min.

Legend for Tables

Table 1 shows the carotenoid composition of cassava root UnB-400 cultivar analyzed in HPLC system. *Trans*- β -carotene was the major carotenoid contributing with 47,17 % of the total content, followed by *cis*- β -carotene isoforms.

Table 2 shows the carotenoid composition of cassava leaves UnB-400 cultivar analyzed in HPLC system. *Trans*- β -carotene was the major carotenoid contributing with 76,57% of the total content, followed by *cis*- β -carotene isoforms

Table 3. Quantification (mg/g of tissue) of lutein, *trans*- β -carotene, *cis*- β -carotene and Retinol equivalents of some cassava cultivars organs.

Figure 1.

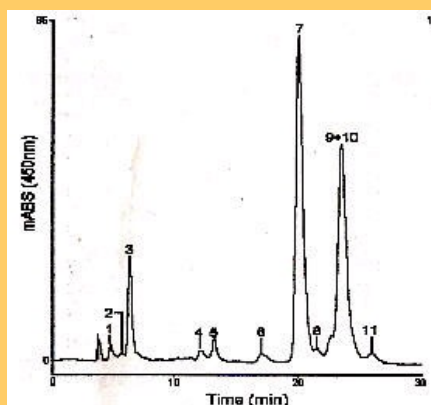


Figure 2.

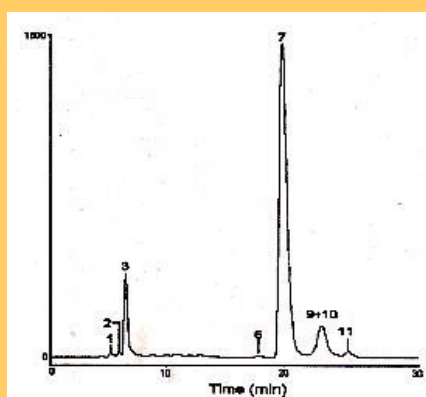


Table 1.

Table 1. Carotenoid composition of cassava root UnB-400 cultivar analyzed in HPLC system. *Trans*- β -carotene was the major carotenoid contributing with 47.17% of the total content, followed by *cis*- β -carotene isoforms.

Peak no. ^a	Carotenoid	T _R (min)	λ_{max}^b (nm)	%
1	Violaxanthin	3.95	376,400,425	0.73
2	5,8-Epoxy-lutein	4.86	404,425,451	0.77
3	Lutein	6.36	420,443,471	8.07
4	β -Cryptoxanthin	12.13	420, 449, 476	0.87
5	<i>cis</i> - β -Cryptoxanthin	13.21	335,420, 445, 470	1.64
6	<i>trans</i> - α -carotene	16.99	423,445,472	1.43
7	<i>trans</i> - β -carotene	20.04	449,479	47.17
8	Phytofluene	22.63	330, 347, 365	1.72
9	9- <i>cis</i> - β -carotene	23.51	446,471	36.59
10	13- <i>cis</i> - β -carotene	23.51	442,469	36.59

^a Numbered according to the chromatogram shown in Fig. 1

^b Obtained with PADD-HPLC system in methanol

Table 2.

Table 2. Carotenoid composition of cassava leaves UnB-400 cultivar analyzed in HPLC system. *Trans*- β -carotene was the major carotenoid contributing with 76.57% of the total content, followed by *cis*- β -carotene isoforms.

Peak no. ^a	Carotenoid	T _R (min)	λ_{max}^b (nm)	%
1	Violaxanthin	4.66	376,400,425	0.5
2	5,8-Epoxy-lutein	5.37	404,425,451	0.53
3	Lutein	5.92	420,443,471	10.16
6	<i>trans</i> - α -carotene	16.25	423,445,472	0.27
7	<i>trans</i> - β -carotene	18.17	449,479	76.57
9	9- <i>cis</i> - β -carotene	21.65	446,471	10.42
10	13- <i>cis</i> - β -carotene	21.65	442,469	10.42

^a Numbered according to the chromatogram shown in Fig. 2

^b Obtained with PADD-HPLC system in methanol

Table 3.

Table 3. Quantification ($\mu\text{g/g}$ of tissue) of lutein, *trans*- β -carotene, *cis*- β -carotene and retinol equivalents of some organs of cassava cultivars.

	Lutein	<i>trans</i> - β -carotene	<i>cis</i> - β -carotene	Retinol equivalent (RE)
Roots				
Cass X Pohli	-	0.16	0.09	0.034
Cass X anomala	-	0.03	0.02	
Cass X pseudog.	-	0.12	0.05	
UnB 101	-	<0.01	< 0.01	
UnB 102	-	0.04	0.03	
UnB 103	-	0.06	0.05	0.286
UnB-400	236.83	1.24	0.96	
UnB 104	-	0.05	0.03	
UnB 105	-	0.08	<0.01	
ICB-300	-	0.19	0.12	0.042
UnB 106	-	0.10	0.08	
UnB 107	-	0.16	0.14	
UnB 108	-	0.06	0.03	
UnB 109	-	<0.01	0.02	
UnB 110	-	<0.01	<0.01	
Leaves				
Cass X anomala	782.15	13.85	2.37	2.505
UnB-400	3081.69	24.12	3.28	4.293
ICB-300	9108.98	18.02	1.88	3.159

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