Application of Near Infra-Red Reflectance Spectroscopy (NIRS) in screening of fresh cassava (*Manihot esculenta*) storage roots for provitamin A carotenoids

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Introduction

Vitamin A deficiency is the most important cause of total blindness in developing countries. It is estimated that 25% of preschool age children in the world has vitamin A deficiency (FAO/WHO 2002). Enhancing the nutritional content of cassava through biofortification will have a significant, positive impact on nutrition status and overall health, especially for poorer communities where cassava is mainly consumed. Several initiatives (HarvestPlus, Agrosalud, SASHA) have been set up to increase the vitamin A concentration in staple food crops to help improve human nutrition status in developing countries. Biofortification is complementary to other strategies for reducing malnutrition like supplementation, fortification, and diversification; nutritional benefits come directly from the biofortified crops with no or little additional costs for consumers. To support biofortification programs there is a need for high throughput techniques to screen macro- and micronutrient concentrations of germplasm and breeding populations in tens of thousands of genotypes in short time frames. High Performance Liquid Chromatography (HPLC) is the common method to determine vitamin A concentration in food crop samples.

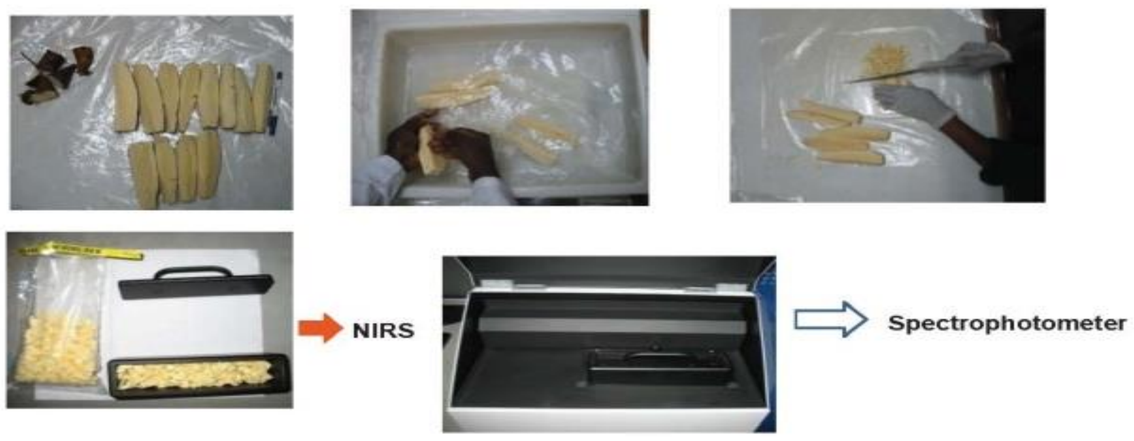
Although HPLC is very accurate, its high costs and the time required for the analysis limit its use to small numbers of samples relative to those required in extensive screening and biofortification programs. Requiring only simple sample preparation methods, NIRS was selected to facilitate the analysis of several traits simultaneously. The potential to estimate vitamin A carotenoid concentrations by NIRS has been demonstrated and applied for example in maize, potato, and Chinese kale (Berardo et al. 2004, Bonierbale et al. 2008, Chen et al. 2009).

Objective

The purpose of this study was to validate NIRS calibrations for analyzing provitamin A carotenoid content of selected, fresh, yellow root cassava genotypes.

Materials and methods

A total of 50 freshly harvested cassava genotypes were obtained in four replications from the experimental fields of IITA in Ibadan, Nigeria. The HarvestPlus Standard method of sampling (Rodriguez-Amaya and Kimura 2004) was employed. Tubers were scanned twice within the range of 400 to 2498 nm registering the absorbance values log (1/R) at 0.5 nm intervals for each sample using the NIRS monochromator (model FOSS XDS, solid module) and coarse cell cups (Fig. 1).The total carotenoid (TC) was determined by the HarvestPlus method (Rodriguez-Amaya and Kimura 2004). Existing NIRS calibration equations were used to predict the β-cryptoxanthin, 13-cis β-carotene, trans β-carotene, 9-cis β-carotene, total β-carotene, and total carotenoid concentrations of the samples.



Results and Discussion

The developed NIRS calibration equations for 13-cis BC, trans BC, 9-cis BC, Total BC and Total Carotenoids showed high coefficients of determination for the calibration curve for chopped cassava samples (0.95, 0.98, 0.88, 0.96 and 0.74, respectively) and medium to high coefficients of determination in cross-validation (0.76, 0.90, 0.69, 0.88 and 0.67, respectively). The standard errors of calibration (SEC) and the standard errors in cross validation (SECV) were low for all traits. Also, the results of carotenoid content of fresh cassava roots of the trials analyzed are presented in Tables 1 to 3. The predicted values found for total carotenoids (TC-spec reference) ranged from 3.93 to 10.51 µg/g with a mean of 7.07 ± 2.55 µg/g for the ICT cassava trial (Table 1), 7.97 to 11.03 µg/g FW with a mean of 9.40 ± 0.76 µg/g for yellow root cassava trial 8 (Table 2), and 6.38 to 10.44 µg/g with a mean of 8.74 ± 1.07 µg/g for yellow root cassava trial 9 (Table 3).

Table 1: Provitamin A carotenoid contents (ug/g) of fresh ICT cassava storage roots predicted by NIRS

Entry	Clone	13-cisBC	bcryptoxanthin	9-cisBC	transBC	TBC	TC	TC_spec
1	I011206	0.79	0.24	0.87	1.86	3.64	3.93	2.57
2	I011371	1.86	0.32	1.63	4.52	8.46	9.59	8.26
3	I061635	2.11	0.33	1.78	4.60	9.26	10.51	5.68
4	I070337	1.00	0.25	1.00	1.89	4.16	5.35	3.02
5	I070374	0.66	0.24	0.85	1.70	3.37	4.40	5.91
6	I070539	1.66	0.33	1.53	4.55	8.02	9.20	9.55
7	I070593	1.58	0.31	1.49	2.75	6.67	9.22	9.97
8	I071378	0.45	0.24	0.69	1.90	3.19	5.04	2.72
9	I071393	0.90	0.26	1.02	2.02	4.61	6.36	3.29
	Means	1.22	0.28	1.21	2.87	5.71	7.07	5.66
	SD	0.59	0.04	0.40	1.30	2.40	2.55	2.99
	SE	0.20	0.01	0.13	0.43	0.80	0.85	1.00
	CV	48.04	14.51	33.11	45.41	42.07	36.06	52.72
	Min	0.45	0.24	0.69	1.70	3.19	3.93	2.57
	Max	2.11	0.33	1.78	4.60	9.26	10.51	9.97

Table 2: Provitamin A carotenoid contents (ug/g) of fresh cassava Yellow root 8 predicted by NIRS

Entry	Clone	13-cisBC	bcryptoxanthin	9-cisBC	transBC	TBC	TC	TC_spec
1	I011368	1.93	0.32	1.61	4.00	8.41	9.43	8.24
2	I011371	1.94	0.31	1.52	4.04	8.54	9.40	7.68
3	I070520	1.32	0.29	1.07	4.97	7.53	8.34	7.22
4	I070536	1.46	0.30	1.34	3.80	7.06	7.97	6.58
5	I070557	1.86	0.30	1.32	3.66	7.75	9.69	7.73
6	I070593	1.98	0.30	1.72	3.99	8.62	9.60	7.86
7	I070670	1.65	0.30	1.38	4.33	7.92	9.30	7.39
8	I070683	1.54	0.29	1.34	5.00	8.45	9.59	6.55
9	I070703	1.67	0.31	1.50	4.61	8.51	9.48	8.23
10	I070736	1.56	0.29	1.40	4.61	8.81	10.32	7.84
11	I070738	1.52	0.30	1.35	5.13	8.47	9.37	8.74
12	I070749	1.49	0.30	1.34	4.71	7.96	8.58	8.43
13	I070753	1.61	0.31	1.51	4.64	8.58	9.53	8.12
14	I071026	2.16	0.33	1.87	4.05	9.37	11.03	7.77
	Means	1.69	0.30	1.45	4.40	8.29	9.40	7.74
	SD	0.24	0.01	0.20	0.47	0.59	0.76	0.64
	SE	0.06	0.00	0.05	0.13	0.16	0.20	0.17
	CV	14.16	3.37	13.59	10.79	7.06	8.10	8.24
	Min	1.32	0.29	1.07	3.66	7.06	7.97	6.55
	Max	2.16	0.33	1.87	5.13	9.37	11.03	8.74

Table 3: Provitamin A carotenoid contents (ug/g) of fresh cassava yellow root 9 predicted by NIRS

Entry	Clone	13-cisBC	bcryptoxanthin	9-cisBC	transBC	TBC	TC	TC_spec
1	I011368	1.67	0.30	1.35	3.40	7.10	8.05	7.35
2	I011371	2.09	0.30	1.63	4.01	8.69	9.87	6.44
3	I082032	1.52	0.30	1.25	3.51	6.83	7.91	6.16
4	I082159	1.50	0.30	1.23	5.99	9.04	9.90	9.65
5	I082189	1.80	0.31	1.41	5.22	9.14	10.18	11.00
6	I082393	1.24	0.30	0.95	4.79	7.00	7.62	7.73
7	I082418	2.09	0.32	1.81	3.10	8.02	9.82	8.54
8	I082425	1.39	0.30	1.24	5.65	8.58	9.10	8.46
9	I082432	1.50	0.30	1.26	4.35	7.77	8.73	7.39
10	I082636	2.21	0.33	1.61	4.24	9.25	10.44	9.12
11	I082708	1.97	0.32	1.61	4.66	8.91	9.91	8.21
12	I082993	1.29	0.28	1.22	2.02	5.15	6.69	7.18
13	I083043	1.99	0.29	1.59	2.74	7.40	8.15	6.05
14	I083196	1.40	0.27	1.10	3.74	7.24	8.51	8.80
15	I083389	1.27	0.26	1.23	2.50	5.58	6.73	4.81
16	I083426	1.75	0.30	1.43	3.72	7.85	8.89	9.36
17	I083535	1.34	0.30	1.14	5.13	8.07	8.60	6.88
18	I083565	1.28	0.29	1.04	5.78	8.56	9.19	4.22
19	I083568	1.54	0.30	1.21	5.04	8.58	9.49	9.10
20	I083579	1.58	0.30	1.28	4.35	7.97	9.01	6.56
21	I083580	1.72	0.31	1.36	4.39	8.03	8.73	8.94
22	I083594	1.26	0.30	1.14	4.47	6.98	8.11	7.21
23	I083718	1.42	0.30	1.18	3.85	6.86	8.97	6.51
24	I083724	1.47	0.31	1.44	3.44	6.89	8.15	6.58
25	I083739	1.43	0.32	1.29	4.74	7.83	9.17	8.12
26	I083774	1.39	0.31	1.38	2.46	5.55	6.38	5.90
27	I083849	1.74	0.30	1.59	4.69	8.99	9.81	8.12
	Means	1.59	0.30	1.33	4.15	7.70	8.74	7.57
	SD	0.28	0.01	0.20	1.04	1.12	1.07	1.54
	SE	0.05	0.00	0.04	0.20	0.22	0.21	0.30
	CV	17.89	4.59	15.31	24.97	14.54	12.26	20.30
	Min	1.24	0.26	0.95	2.02	5.15	6.38	4.22
	Max	2.21	0.33	1.81	5.99	9.25	10.44	11.00

The corresponding total carotenoid results using the reference spectrophotometric method (TC-spec) were found to range from 2.57 to 9.97 µg/g with a mean of 5.66 ± 2.99 µg/g for the ICT cassava trial, 6.55 to 8.74 µg/g with a mean of 7.74 ± 0.64 µg/g for yellow root cassava trial 8, and 4.22 to 11.00 µg/g with a mean of 7.57 ± 1.54 µg/g for yellow root cassava trial 9. There is significant ($P < 0.001$) positive correlation (r = 0.55) between TC-predicted by NIRS and TC-spec. There is also significant ($P < 0.001$) positive correlation (r = 0.52) between *trans* β-carotene predicted by NIRS using HPLC reference and TC-spec (Fig. 2 and Table 4).

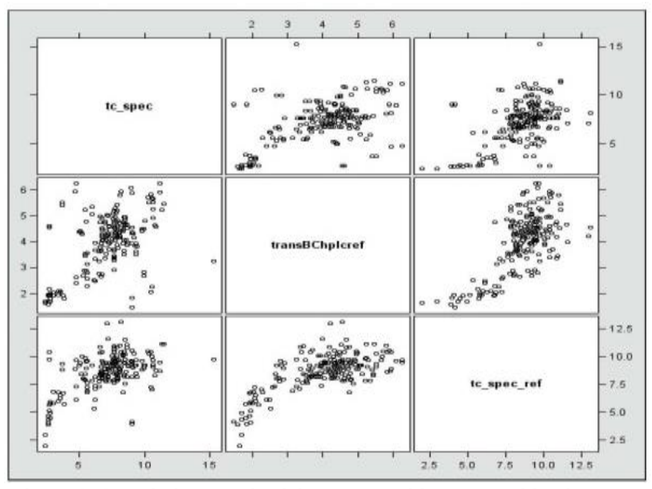


Figure 2. Correlation graph of the carotenoid content of fresh cassava storage roots using NIRS

Table 4: Table of Pearson correlation coefficients of carotenoid content of fresh cassava storage roots using NIRS

Pearson Correlation Coefficients			
Parameters	TC_spec	transBC(NIRS)	TC_(NIRS)
TC_spec	1.00000	0.52049***	0.55010***
transBC(NIRS)	0.55010***	1.00000	0.69507***
TC_(NIRS)	0.55010***	0.69507***	1.00000

Conclusions

This study shows that the developed NIRS calibration equations can be used to predict total carotenoids and trans β-carotene content of yellow root cassava and can serve as a fast and cost-effective method for screening large sample sizes by cassava breeding programs.

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