Decrease in β -Carotene Content of Yellowish Cassava Roots Suffered from Physiological Deterioration†

Lydia A. Gloria* & Ikuzo Uritani**

* Department of Agricultural Chemistry and Food Science, Visayas State College of Agriculture (ViSCA), Baybay, Leyte 7127-A, Philippines

** Nodai Research Institute, Tokyo University of Agriculture, Sakuragaoka, Setagaya-ku, Tokyo 156, and Faculty of Home Economics, Nagoya Women's University, Shioji-cho, Mizuho-ku, Nagoya 467

Almost all the orange pigment in the yellowish roots of cassava (cv. Golden Yellow) was indicated to be β -carotene. The content of β -carotene in the tissue was mostly in the range of 10 to 70 μg per g dry weight, and was largest in the innermost part (C-part) per g dry weight, second in the intervening part (B-part, where PD occurred), and smallest in the outermost part (A-part). The content in the B- and C-parts decreased following the appearance and development of PD.

Root flesh of some cultivars of cassava shows orange color. At first we tried to investigate whether the orange color in the roots of a cassava plant called cv. Golden Yellow, a common variety in Leyte, was based on β -carotene, a provitamin A. During the experiments, we were informed of the finding* of the Food and Nutrition Research Institute (FNRI), NSTA, Manila to identify the orange pigment in Golden Yellow cassava roots with β -carotene. On the other hand, harvested cassava roots are perishable physiologically and this is called physiological deterioration (PD)¹⁾⁻⁵⁾ showing many brownish strings in the intervening part⁴⁾ (called the B-part) of the parenchymatous tissue between the outermost tissue (called the

A-part) and the innermost tissue (called the C-part). Our observation by the naked eye suggested that the intensity of the orange color in the parenchymatous tissue of Golden Yellow roots decreased following the appearance and development of PD in the B-part.

This paper deals with the confirmation of the orange pigment in the roots to be β -carotene and with the decrease in β -carotene content in the parenchymatous tissue in Golden Yellow cassava roots in relation to PD.

1. Sampling of root tissue

About 8 month old cassava (*Manihot esculenta* Crantz, cv. Golden Yellow, yellowish flesh, sweet type) roots were harvested at the farm of PRCRTC, ViSCA, and used for the analysis immediately after harvest or after incubation for one to several days under room conditions (about 26° to 30°C and at relative humidities of 78 to 92%). Each root was washed with tap water and cut perpendicularly to the axis into tissue blocks (1 to 2 cm thick), and the severity of PD in the tissue was evaluated by the numerical score of biochemical grade, designed in the previous paper⁴⁾. That is, No. 0: no symptoms to the naked eye under a UV-lamp; No. 1: bluish fluorescence under a UV-lamp (due to the presence of the coumarin derivatives)⁸⁾, but no symptoms to the naked eye; No. 2: faint bluish coloration to the naked eye (the tissue cells are surviving); No. 3: light-brown streakings with morphological changes; No. 4: moderate-brown streakings; and No. 5: severe-brown streakings. As described previously⁴⁾, some difference in the biochemical grade was often seen between the upper and lower halves of the root. Furthermore, it was sometimes observed that the biochemical grade was different dependent upon the position in a cut surface of one tissue block. Thus, sampling of root tissue was made according to the biochemical grade of PD, and with or without separation of tissue blocks into the A-, B- and C-parts (see

† This work was implemented at the Philippine Root Crop Research and Training Center (PRCRTC), ViSCA, Baybay, Leyte under the Grants on Overseas Scientific Survey of the Ministry of Education, Science and Culture, Japan (MESCJ), and on the Scientific Cooperation Program between the National Science and Technology Authority (NSTA, Philippines) and the Japan Society for the Promotion of Science (JSPS, Japan).

* Personal communication by Dr. Josefina Bulatao-JAYME, FNRI, NSTA.

Table 2).

2. Carotene analysis

β -Carotene content was determined by WORKER's method⁶⁾ with minor modifications. The carotenes were extracted using wet and dried tissues. In the wet method, 2 g of chopped pieces of each cassava tissue was homogenized in 5 ml acetone, and the homogenate was then centrifuged. The supernatant solution containing the pigment was left in a beaker at room conditions in the dark to evaporate acetone. Then, the residue was dried in the dark in a desiccator with H_2SO_4 . In the dry method, the above chopped pieces was dried overnight at 60°C, then ground. One g of the sample was homogenized in acetone, the suspension filtered, and the filtrate containing the pigment was dried in the same way as in the wet method.

The dried residue was dissolved in *n*-hexane or petroleum ether, the solution passed through an Al_2O_3 column. The carotene adsorbed on the top was eluted from the column with 2% acetone in *n*-hexane or in petroleum ether. The content of β -carotene in the eluate was determined in a spectrophotometer using authentic β -carotene (Sigma, grade Type III) as the standard.

3. Results and Discussion

It must be disclosed first that it was common to observe that the content of β -carotene in the tissue varied widely from root to root even though the roots were taken from the same plant, and that the proportion of the A-, B- and C-parts in one root differed widely from the basal to the distal part of the root.

(1) Identification of the orange pigment with β -carotene

All of the orange pigment in the root (cv. Golden Yellow) was extracted by acetone, dissolved in *n*-hexane or petroleum ether, adsorbed on the top of the Al_2O_3 column and eluted by 2% acetone in *n*-hexane or in petroleum ether. The results indicated that all of the pigment belonged to carotenes.

The separated pigment, collected from the Al_2O_3 column using 2% acetone in *n*-hexane, was read in the range of 380 to 570 nm in a spectrophotometer with a clear peak of the

Table 1 The β -carotene content in the A-, B- and C-parts of freshly harvested roots

Samples*	Parts	Carotene (μ g/g dry wt.)
I	A	trace
	B	15.90
	C	34.09
II	A	7.79
	B	10.34
	C	45.17

* I and II: Extracted by the wet method using *n*-hexane and the dry method using petroleum ether, respectively.

adsorption spectrum at 450 nm, which coincided with that of the authentic sample. The sample read in the IR spectro-photometer gave a spectrum comparable to that of the authentic sample. Thus, almost all the pigment in the root was regarded as β -carotene.

(2) Carotene content in different tissue parts

Table 1 shows the β -carotene content in the A-, B- and C-parts in the parenchymatous tissue of freshly harvested roots. One can see that the carotene content increases from the outermost part (A-part) to the innermost part (C-part) of the parenchymatous tissue.

(3) Decrease in β -carotene following physiological deterioration

Four sets of data on the changes in β -carotene content following PD are presented in Table 2. These experiments were done at different times, using varying methods of analysis as indicated above. It was seen that the occurrence and severity of PD in the roots varied widely from root to root, as described previously⁴⁾. For example, PD occurred 2 days after harvest in one case, but appeared 9 days after harvest in another case. However, it was visually observed that PD was always accompanied by a loss in the orange color. This was supported by the quantitative analysis for β -carotene (Table 2). That is, the decrease in β -carotene paralleled the biochemical grade (severity) of PD. Thus, we understand that the loss in the orange color in relation to PD was due to the

Table 2 β -Carotene content in the tissue parts of different biochemical grades of physiological deterioration

Experiments*	Tissue parts	Biochemical grades of PD	Carotene ($\mu\text{g/g}$ dry wt.)
I	A	3	8.26
	B	5	3.55
	B	2	42.36
	C	2	69.78
II	A	2	9.75
	B	3	17.97
	B	2	34.13
	C	1 (without visual PD)	73.44
III	BC**	2	20.04
	BC	4	4.60
	BC	4	8.83
	BC	5	3.64
	BC	5	5.55
IV	ABC**	1 (without visual PD)	51.66
	ABC	2	38.33
	ABC	3	18.29
	ABC	4	15.98
	ABC	5	11.13
	B	4	9.33
	B	5	7.77
	C	4	10.66
	C	5	6.22

* I, II, III and IV: Experiments I and II were conducted in August, 1982, by the dry method with *n*-hexane; Experiment III was done in January, 1983, by the dry method with petroleum ether; Experiment IV was performed in March, 1983, by the wet method with *n*-hexane.

** Parenchymatous tissues containing the B- and C-parts, and the A-, B- and C-parts.

decrease in β -carotene content of the roots.

An important problem to be attended is storage of cassava roots in the fresh condition after harvest, as the roots are susceptible to PD, which is characterized by brownish discoloration, rendering the roots unsuitable as food. Furthermore, it should be noted that β -carotene content decreased following the appearance and development of PD, especially in the B-part (where PD occurred) and the C-part. We have not yet analyzed exactly whether or not the content in the A-part decreased in parallel with PD occurrence. We think that the decrease in β -carotene content in the B- and C-parts should not be overlooked

from the nutritious point of view, considering that the main portion of the parenchymatous tissue composes B- and C-parts. Cassava roots are also susceptible to microbial deterioration (MD) which is caused by the infection of postharvest fungi⁽⁷⁾⁽⁸⁾ such as *Botryodiplodia theobromae*, *Aspergillus flavus*, *Rhizopus* sp. and *W-fungus*⁽⁹⁾. We assume that β -carotene content also decreases in the course of MD development. MD may cause not only the loss of β -carotene and other nutritious constituents but also the contamination with mycotoxins produced by the postharvest fungi. In fact, it was reported that cassava roots were sometimes contaminated with aflatoxins, the strong

carcinogenic mycotoxin of *A. flavus*⁹⁾.

It is of much significance to discuss the mechanism on the loss of β -carotene in PD-induced roots. As mentioned above, β -carotene is inclined to be oxidatively decomposed indirectly by lipoxygenase and polyphenol oxidase in the presence of the substrates such as linolenic acid and polyphenols, respectively¹⁰⁾¹¹⁾, and directly by a β -carotene-bleaching enzyme which was proven to exist in tea leaves¹¹⁾. Polyphenols such as (+)-catechin³⁾ were produced in parallel with PD occurrence, and polyphenol oxidase activity was present in either fresh or deteriorated tissue⁴⁾⁷⁾¹²⁾. It may also be possible that either lipoxygenase or a kind of β -carotene-bleaching enzyme is produced in parallel with the appearance of PD. It would be useful to study the mechanism, considering that the loss of β -carotene is caused in the C-part where PD is not induced, as is caused in the B-part, the area of PD occurrence.

Acknowledgements: The authors express their sincere thanks to Dr. Emma S. DATA, PRCRTC, ViSCA for extending to them the necessary convenience in the performance of the experiments. They are indebted to Professor A. GERFACIO, Department of Animal Science, University of the Philippines at Los Baños, College, Laguna, for providing them with authentic β -carotene. They are grateful to Miss Marieta A. GORGONIO for actively assisting them in the experiments.

References

- 1) BOOTH, R. H.: *Exptl. Agric.*, **12**, 103 (1976).
- 2) LOZANO, L. C., COCK, J. H. and CASTANO, J.: *CIAT Series CE-14*, 135 (1978).
- 3) TANAKA, Y., DATA, E. S., HIROSE, S., TANIGUCHI, T. and URITANI, I.: *Agric. Biol. Chem.*, **47**, 693 (1983).
- 4) URITANI, I., DATA, E. S., VILLEGAS, R. J., FLORES, P. and HIROSE, S.: *Agric. Biol. Chem.*, **47**, 1591 (1983).

- 5) HIROSE, S., DATA, E. S. and URITANI, I.: *Japan. J. Trop. Agr.*, **27**, 149 (1983).
- 6) WORKER, A. A.: *J. Sci. Food Agric.*, **8**, 442 (1957).
- 7) RICKARD, J. E. and COURSEY, D. G.: *Trop. Sci.*, **23**, 1 (1981).
- 8) TANIGUCHI, T. and DATA, E. S.: Bio-resources Investigation of Production, Storage, Processing and Vegetation of Root Crops in the Tropics, 1981 Interim Report (I. URITANI and E. D. REYES eds.), Nagoya Univ. Coop., Nagoya, p. 22 (1982).
- 9) BULATAO-JAYME, J., ALMERO, E. M., CASTRO, M. C. A., JARDELEZA, M. T. R. and SALAMAT, L. A.: *Internl. J. Epidemiology*, **11**, 112 (1982).
- 10) ESKIN, N. A. M., HENDERSON, H. M. and TOWNSEND, R. J.: *Biochemistry of Foods*, Academic Press, New York and London, p. 150 (1971).
- 11) KATO, M., OMORI, S., OBATA, Y., SAIJO, R. and TAKEO, T.: Abstracts of Papers, Annual Meeting of Agric. Chem. Soc. Japan, Sendai, March, p. 227 (1983).
- 12) RICKARD, J. E., MARRIOTT, J. and GAHAN, P. B.: *Ann. Bot.* **43**, 523 (1979).

(Received Feb. 9, 1984)

生理的変質を受けた黄色キャッサバ塊根の β -カロチン量の減少

リディア・A・グロリア*, 瓜谷郁三**

(* ビザヤ国立農科大学農芸化学及び食品科学科)

** 東京農業大学総合研究所及び名古屋女子大学家政学部)

キャッサバ (品種: ゴールデンイエロー) の黄色塊根中のオレンジ色の色素はほとんど β -カロチンであることがわかった。組織中の β -カロチン量は乾物 1g 当たりおおよそ 10~70 μ g の範囲にあり, その量は乾物 1g 当たりにして最内層 (C-部位) において最も多く, 次に中間層 (B-部位, 生理的変質が起こる部位) であり, 最外層 (A-部位) において最も少なかった。B 部位や C 部位における含量は生理的変質の発現と激しさに対応して減少した。