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メタデータ	言語: eng 出版者: 公開日: 2007-04-25 キーワード (Ja): キーワード (En): 作成者: YAMAMOTO, Yoshio, TOMITA, Yuichiro メールアドレス: 所属:
URL	<a href="http://hdl.handle.net/10232/3182">http://hdl.handle.net/10232/3182</a>

## Studies on the Bio-Pigments and Vitamins:

### V. Correlative Changes among the Carotene, Total Carotenoids and the Other Constituents of Sweet Potatoes in relation to the Variety and Storage (2)

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#### Introduction

In the previous paper,<sup>(1)</sup> the authors have reported the correlative changes among the carotene, total carotenoids, moisture and starch content of sweet potatoes in relation to the variety and storage.

Studies reported in this paper include experiments on the correlative changes among the carotene, total carotenoids and the water soluble vitamins (B<sub>1</sub>, B<sub>2</sub> and C) content in relation to the variety and storage.

#### Materials and Methods

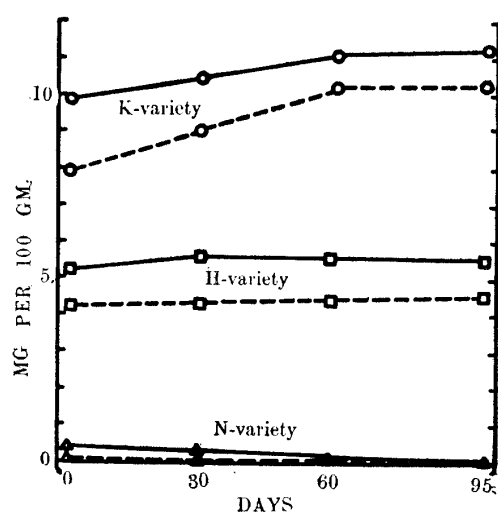
Three varieties of sweet potatoes were used in this study: Kagoshima 7-1061, Hayato and Nōrin No. 2, designated K-, H- and N-variety respectively.

The methods and general procedure used in this study, storing and sampling etc., were the same as previously described.<sup>(1)</sup>

The determination of the water soluble vitamins was carried out as follows: the vitamin B<sub>1</sub> was determined according to the thiochrome method<sup>(2)</sup> and the vitamin B<sub>2</sub> according to the fluorometric method modified by the authors.<sup>(3)</sup> Total- and L-ascorbic acid content were determined using the colorimetric method with 2,6-dichlorophenol indophenol.<sup>(2)</sup>

#### Results

The results showing changes in the carotene and total carotenoids content of three varieties of sweet potatoes during storage are given in Fig. 1, where it is evident that these pigments content was the highest in K-variety, an intermediate in H-variety and the lowest in N-variety, not only at the harvest time but also at any stage in storage. Although two varieties of K and H increased these pigments content during storage, only N-variety caused a pronounced decrease; the percentages of increase in total carotenoids and carotene of K-variety after three months of storage were 11.6 and 31.7% respectively, and those of H-variety 1.5 and 8.1%, whilst N-variety had lost 70% in total carotenoids and 86% in carotene during the same period. Therefore, it may be said that



—TOTAL PIGMENTS. ....CAROTENE.

Fig. 1. Changes in the carotene and total carotenoid pigments of sweet potatoes during storage.

the variety of sweet potatoes is also an important factor in determining changes in the carotenoid or carotene levels during storage. As shown in Fig. 1 and the previous report,<sup>(1)</sup> it is also of most practical importance from the nutritional viewpoints that the post-harvest increases in the carotenoids content are mainly due to  $\beta$ -carotene, the pro-vitamin A.

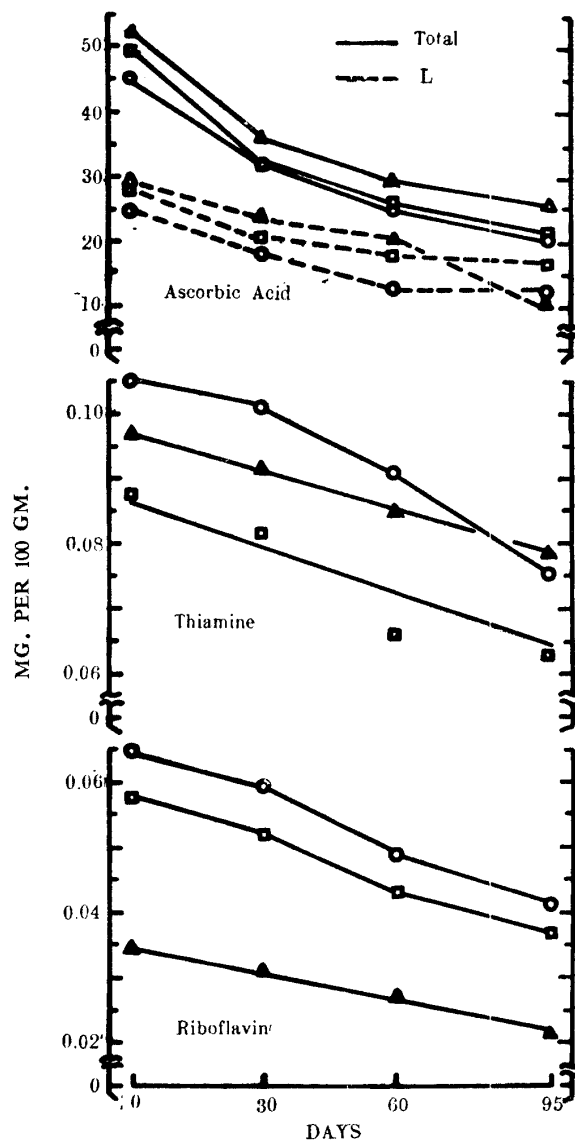
Simultaneously a study was performed on changes in amount of water soluble vitamins (vitamin B<sub>1</sub>, B<sub>2</sub> and ascorbic acid) during storage and the results are shown in Table 1 and Fig. 2. From the results obtained, all the vitamins tested in this study showed a tendency to decrease in amount during storage. Furthermore, when observed on each vitamin tested, it may be stated as follows:

Table 1. Changes in Ascorbic Acid, Thiamine, Riboflavin Content and Their Ratios of Remains of Sweet Potatoes during Storage.

Vitamin examined	Variety	Storage Times (Days)								
		0		30		60		95		
		mg%	%	mg%	%	mg%	%	mg%	%	
Ascorbic acid	Total	Nōrin No. 2	52.9	100	35.6	67.28	30.1	56.89	25.5	48.20
		Hayato	49.5	100	32.3	65.04	26.0	52.52	22.1	44.64
		Kagoshima 7-1061	45.7	100	32.2	70.52	24.8	54.31	20.9	45.77
	L-	Nōrin No. 2	29.1	100	23.9	82.22	21.2	72.93	10.5	36.12
		Hayato	28.5	100	21.2	74.41	18.0	63.18	17.1	60.02
		Kagoshima 7-1061	25.2	100	18.1	64.62	12.9	46.05	12.7	45.34
Thiamine	Nōrin No. 2	r%	%	r%	%	r%	%	r%	%	
	Hayato	97.1	100	91.8	94.55	85.1	87.65	78.9	81.27	
	Kagoshima 7-1061	88.2	100	82.3	93.00	66.5	75.15	62.8	70.96	
Riboflavin	Nōrin No. 2	105.5	100	101.0	95.84	91.3	86.55	75.2	71.29	
	Hayato	r%	%	r%	%	r%	%	r%	%	
	Kagoshima 7-1061	34.5	100	30.4	88.16	26.9	78.01	21.8	63.22	
Hayato	57.5	100	52.6	91.52	45.1	78.47	37.2	64.73		
Kagoshima 7-1061	64.6	100	59.4	91.92	49.0	75.95	41.1	63.71		

(a) The total ascorbic acid content of the three varieties showed a significant decrease early in the storage period (during the first month of storage) and then decreased gradually. After three months of storage, all the varieties had lost about half as much ascorbic acid as that they had at harvest. The K- and H- variety richest in carotene, however, showed a tendency to maintain L-ascorbic acid content from the intermediate period (about two months) to the end in storage. In this connection, it must be also emphasized that with respect to the varieties of the sweet potatoes, the higher the carotene content, the lower the ascorbic acid content not only at harvest but also at any stage in storage.

(b) Except for K- variety showing more decrease in the vitamin B<sub>1</sub> content at the end in storage than did N- variety, this vitamin content decreased in the order of K-, N- and



○ Kogoshima 7-1061. □ Hayato. △ Nōrin No 2.

Fig. 2. Changes in ascorbic acid, thiamine and riboflavin content of sweet potatoes during storage.

H-variety from the harvest to the end in storage. Such the behaviour observed on this vitamin can not be considered as the varietal differences. And therefore, it may be suggested that there is no relationship between the carotene production and the vitamin B<sub>1</sub> content during storage.

(c) On the contrary, a correlation due to the variety of sweet potatoes was found between the carotene and the vitamin B<sub>2</sub> content; namely, the vitamin B<sub>2</sub> content of K-, H- and N- variety at harvest were 64.6, 57.5 and 34.5  $\gamma$  %, respectively and after three months that of the three varieties decreased to 41.1, 37.2 and 21.8  $\gamma$  % respectively, as shown in Table 1, although the ratios of remains were about 63.7, 64.7 and 63.2 % respectively and little apparent differences had been found from this point of view. Such the varietal differences of the riboflavin content in sweet potatoes was the same as observed in the case of carotene as described above and it is very interest that the varieties rich in carotene are also more rich in the vitamin B<sub>2</sub> content than those poor in carotene.

### Discussion

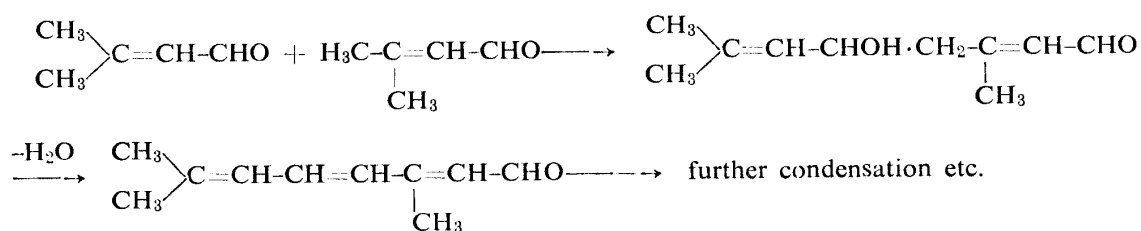
Tomita<sup>(4)</sup> has reported that there was a tendency for the variety of sweet potatoes containing high starch content to be also high in ascorbic acid content. According to the previous publication,<sup>(1)</sup> it was found that there was a tendency for the starch content to decrease in the order of N-, H- and K- variety and the same was true of ascorbic acid as shown in Fig. 2, although K- variety contained more ascorbic acid than did the other two varieties on the dry matter basis. Giroud<sup>(5)</sup> maintains that carotene has an antioxidant function rather than a pro-oxidant action; he claims that plants richest in carotenoids are also richest in ascorbic acid, for this vitamin is protected by the carotenoids. There is, however, no real evidence that this suggested correlation between vitamin C and carotenoid levels is a reality.<sup>(6)</sup> Thus, various opinions are divided in this problem, but in our experimental conditions there found a reverse correlation between the total carotenoids or  $\beta$ -carotene and the ascorbic acid content of sweet potatoes. If this finding would be true, it may be interpreted that the carotenoids are protected by the ascorbic acid in contrast with Giroud's opinion.<sup>(5)</sup> However, further investigations are required to elucidate this problem.

In regard to the relationship between the carotenoids and the vitamin B<sub>1</sub> content in plants, Pfützer et al.<sup>(7)</sup> state that barley seeds rich in nitrogen and aneurin produce plants giving a greater yield of carotenoids than do seeds less rich in these constituents. However, Luteraan et al.<sup>(8)</sup> argue in this respect that when an unspecified *Penicillium* is cultured on a medium completely free from thiamine, it produces a considerable amount of carotenoids in place of the usual non-carotenoid pigments. Contrary to the result just mentioned above, Lutz<sup>(9)</sup> has reported that in two paratuberde bacilli (e. g. *B. lambardo-pellegrini* and *B. boquet*) the degree of pigmentation (presumably mainly due to carotenoids) is directly proportional to the thiamine content of the medium. And moreover, a following quantitative data have been reported as to the synthesis of  $\beta$ -carotene in *Phycomyces blackesleanus*, in which it was inhibited in media containing less than 0.2  $\gamma$  thiamine per 100 ml.<sup>(10)</sup>

As just stated, various opinions are divided with regard to the relation between the carotenoids production and the vitamin B<sub>1</sub> levels in the biological materials. Such the

variations would be mainly due to the various facts that the carotenogenesis in plant materials was carried out *via* very complicate processes and a difference due to species between the higher plants and the micro-organisms may cause a varied mode of carotenoids production and furthermore, contradictory results were caused by the difference of the experimental conditions etc. In the authors' experiments, K-variety richest in carotene was also richest in vitamin B<sub>1</sub> at any storage period except the later stage of storage, but N-variety lowest in this pigment had much more thiamine content than did H-variety containing the intermediate carotene, as shown in Figs. 1 and 2. Judging from these findings, it may be presumably given as conclusion that there is no obvious accuracy on the correlative relationship between the carotenoid production and the thiamine content.

Moreover, in the relation to this subject, the authors have examined the relationship between the carotenoid increase and the vitamin B<sub>2</sub> content of sweet potatoes during storage. Up to now, very little work on this problem has been reported. There is only an investigation reported by Goodwin et al.<sup>(11)</sup>, where in the cultures of *Phycomyces blacklesleanus* riboflavin up to a concentration of 1/20,000 stimulates growth (dry weight not lipid), lipogenesis and carotenogenesis; at higher concentrations a marked inhibition of all three processes occurs. In our experiments, as shown in Fig. 2, K-variety contained the highest riboflavin content at harvest as well as at any stage in storage, H-variety an intermediate and N-variety the lowest. This order remained unchangeable at the basis of dry matter; in our experimental conditions, a positive correlation due to the variety of the sweet potatoes was clearly recognized between carotene production and riboflavin level, differing from the results obtained in the case of thiamine. This finding will support the suggestion that the vitamin B<sub>2</sub> plays an important part in an enzymatic function for the carotenoids biosynthesis. Because, it is well known that the riboflavin takes the first place in the biological oxidation-reduction as the component of FAD or FMN (coenzymes of yellow enzymes) and furthermore, with the aid of the present knowledge, active acetate derived from the carbohydrates is changed in C<sub>5</sub> unit (e. g.  $\beta$ -methylcrotonaldehyde, mevalonic acid etc.) *via* TCA- and fatty acid-cycle, and subsequently C<sub>5</sub> unit thus produced may be the repeating unit, the synthesis of C<sub>40</sub> carotenoids being brought by an aldol type condensation followed by removal of water.<sup>(12-13)</sup> The schema would be briefly shown as follows:



Recently, mevalonic acid is considered as a precursor not only for cholesterol,<sup>(14)</sup> squalene,<sup>(15-16)</sup> rubber,<sup>(17)</sup> and  $\beta$ -carotene<sup>(18)</sup> but for lycopene<sup>(19)</sup> also. Judging from these possible assumptions, in either case, it is not too much to say that the fundamental unit for the carotenogenesis can be derived from both TCA- and fatty acid-cycle. In fact, the occurrence of citric and malic acid etc., intermediates of TCA-cycle, is not only well known in various plant tissues,<sup>(20)</sup> but also the occurrence of these acids has been recognized in the sweet potato tubers.<sup>(21)</sup> These acids may be acted by dehydrogenases possessing DPN or TPN as the coenzyme, followed by the flavin enzymes. Such being the case, it

should be emphasized that the enzymatic significance for riboflavin to play a part in C<sub>5</sub> unit production is very important. From this point of view, it may be probably considered that carotene production is closely connected with riboflavin content in sweet potatoes, but the full explanation of this must await further work.

#### Acknowledgements

The authors wish to express their sincere gratitude to Dr. K. Kondo for his advice and criticism given throughout this investigation, and also are grateful to Prof. S. Nakamura and Mr. S. Shimoōsako of this faculty for the supply of sweet potatoes.

#### Summary

The correlative changes among the carotene, total carotenoid pigments and water soluble vitamins (B<sub>1</sub>, B<sub>2</sub> and C) were examined during the period in storage.

Three varieties of the sweet potatoes were used; Kagoshima 7-1061 (K-variety), Hayato (H-variety) and Nōrin No. 2 (N-variety).

The results obtained may be summarized as follows:

- (1) Both carotene and total carotenoids content were the highest in K-variety, an intermediate in H-variety and the lowest in N-variety not only at harvest but also at any stage in storage. K- and H-variety caused a real increase in both carotene and total carotenoids content during storage in our experimental conditions; K-variety caused an increase in carotene and total carotenoid content as much as 31.7 and 11.6% respectively, H-variety 8.1 and 1.5%, whilst only N-variety caused a decrease as much as 86 and 70% respectively during the same period as compared with that for the harvest.
- (2) Most of the thiamine, riboflavin and ascorbic acid content of the three varieties showed a decrease during storage.
- (3) A tendency was observed that the variety containing the higher carotene content possessed reversably the less ascorbic acid content, both at harvest and at any stage in storage.
- (4) In the case of changes in vitamin B<sub>1</sub> content during storage, there found no varietal consistency, differing from the results obtained in the case of carotene and ascorbic acid.
- (5) On the contrary, a positive correlation due to the variety of sweet potatoes was clearly recognized between carotene increase and riboflavin levels during storage; the variety richest in carotene had also the highest riboflavin content.

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