same plant, it is generally high in those over medium size. Between the roots of different plants, however, individual differences are eminent even if they are of same size. (3) According to the results of water-culture, the carotene content of sweet potato is influenced with plant nutrients. Above all, it is eminent in the case of phosphoric acid, that is, when much phosphoric acid is given the total yield of roots surely increases, nevertheless the carotene content of individual root remarkably decreases. (4) The carotene content of raw sweet potato exceedingly increases during the storage period. Young plants of sweet potato, sprouted from the same root, were water-cultured under the same conditions, and thus cropped sweet potatoes of almost equal carotene content were stored in a repository. In the consequences, the increasing ratio of carotene amounted to 50% after two months and moreover, 75% after four months. (5) At the time when sweet potato germinates, the reduction of carotene remains excessively minute. Especially when sunbeam is intercepted while germination, the reduction becomes further minute. (6) The destruction of sweet potato-carotene on account of steaming or boiling with rice is very low, and even in the case of these treatments more than 90% of carotene remains.

Knowing these facts in the previous studies, the author and his collaborators successively have continued the studies on the stability of sweet potato-carotene. The authors will report herein on the summary of the results thus obtained since.

## II. Factors Influencing the Destruction of Sweet Potato-carotene

Kôtaro NISHIDA and Masakuni ICHIKI

As destroying factors against the carotene in plants, we can count up those such as heat, light, oxygen, temperature, moisture and enzyme etc. As regards to the weights of these factors, however, investigators have been fully differing in their opinions and in an extreme case even direct opposite results have been reported.

In a review by Lantz,[8] when pepper was dehydrated its carotene from 55 to 65% was destroyed, while when dried in the sun more than 90% was lost. But Sherman *et al.*[9] mentioned that there were scarcely any difference in the ratio of carotene-destruction either in the sun dried sweet potato or in the dehydrated. According to J. H. Mitchell *et al.*[10] though the sweet potato flour lost 89% of its carotene

after 8 months' sealed storage, when stored in inert gas or in vacuum the destruction of carotene remained only in 17 to 24% even after one year. Mallette *et al.*[11] learned the remarkable effect of storing temperature. Chase[12] reported that the carotene was stable when the moisture of sweet potato flour was less than 5%.

As to the enzyme participating in carotene-destruction, first, Miller *et al.*[13] mentioned that, in the case of not perfectly blanched dry sweet potato, the inactivation of enzymes was not sufficient, and thus it became the greatest cause of carotene-destruction. Mallette *et al.*,[11] however, concluded that between the stability of carotene and various oxidation enzymes there were not so dependent relations to each other. By Sherman *et al.*,[9] moreover, in the blanched sweet potato, the destruction ratio of carotene rather remarkably increased during the dehydration process.

Bernstein *et al.*[14] reported on the enzymatic destruction in the studies on the decomposition of carotene. More recent studies by H. L. Mitchel *et al.*[15] showed that the destruction of carotene, which occurred during the storage of alfalfa, was due to lipoxidase which was inactivated with heating process proceeded to drying. This enzyme — lipoxidase — had been discovered in soy bean by Bohn and Haas[16]* and investigated by Sumner and Dounce,[17] Sumner *et al.*,[18] Tauber,[16] Mikhlin *et al.*,[19,20,21] Theorell *et al.*,[22,23] and Fukuba.[24,25]

As above mentioned, on the destructive factors of sweet potato-carotene the definite conclusion has nowadays not yet been acquired, and therefore the authors took up this problem as the first investigation.

## Experimental results and discussion

### A. The relations between the stability of carotene and the treatment of sweet potato

a) Experimental method: In order to get as homogeneous sample as possible, a very big carotene-sweet potato (880 gm.) was lengthwise divided in two; one of them was immediately mashed and then dried in the sun or with electrical heat, the other was further broadwise divided in two, mashed after being blanched for 30 minutes, and then dried in the sun or with electrical heat.

In the sun-dried sample which was dried in the direct sun for 3 days, its carotene contents were estimated twice, respectively after 5 and 15 days' storage in a desiccator. The electric heated sample, that

* cited in Tauber's report

was dried in an electric dryer at 60° to 80°C for 3 days was immediately stored in a colored desiccator to avoid the effect of light, and 5 days later its carotene content was determined. Further, 5 days later ultraviolet ray was irradiated on it and the carotene-destruction due to light, was measured. Ultraviolet ray was irradiated for 30 minutes on it kept at 25 cm. from a mercury lamp (voltage: 100 V., current: 8 A.).

In order to reject the influences of oxygen and light, apart from the above sample, the raw and the blanched mash was put into a colored desiccator and the air was evacuated, and $CO_2$ gas was filled twice everyday. In this way 10 days afterwards carotene content was estimated.

In all the experiments, Willstätter and Stoll's method[26] was adopted in estimating the carotene content, the results being shown in total contents of carotene.

b) Experimental results: The results of above mentioned experiments are presented in Table 1.

Table 1.

The relations between the treatment of sweet potato and the stability of its carotene

| Sample | Days of storage | Raw mash | | Blanched mash | |
|---|---|---|---|---|---|
| | | Carotene γ% (in dry matter) | Ratio of remains % | Carotene γ% (in dry matter) | Ratio of remains % |
| Original | 0 | 17240 | 100 | 17240 | 100 |
| Sun-dried | 5 | 13410 | 78 | 14580 | 85 |
| 〃 | 15 | 7810 | 45 | 12890 | 75 |
| Electric heated | 5 | 15600 | 90 | 15980 | 93 |
| Ultraviloet ray irradiated | 10 | 11120 | 65 | 12580 | 73 |
| Stored in $CO_2$ gas | 10 | 16710 | 97 | 14850 | 86 |

The results in Table 1 can be summarized as follows:—

(1) More carotene was lost with sun-drying than with dehydration. (2) Carotene was greatly decomposed under the irradiation of ultraviolet ray. (3) In all the samples, the ratio of carotene-remaining was higher in the flour made of blanched sweet potato than in those of not blanched. The sample stored in $CO_2$ gas, however, was the only exception.

c) Discussion: The influence of light on the destruction of carotene was evident in the authors' experiments, and on this point, differing

from Sherman's[9] results, the authors proved the same tendency as Lantz[8] had gotten.

As for the action of ultraviolet ray, Luccetti[27] recognized that carotene was oxidatively decomposed when 2 mm. layer of butter was irradiated for 8 hrs. in the air. Further, by Sreenevasson *et al.*,[28] the exposure to the irradiation of ultraviolet ray for 2 hrs. had equal effect as of the diffused ray for 6 months. In the authors' experiments, too, it was shown that carotene was greatly destructed on account of ultraviolet ray.

Though Sherman[9] said that the blanching process rather increased the degradation of carotene, the authors got such results, coinciding with the studies by Miller[13] or H. L. Mitchel *et al.*,[15] that this process effectively prevented the degradation. As appropriate method of determining the time for the blanching, it is considered today as expediently adequate to aim at the time needed for making negative the reaction of thermostable peroxidase.[29] In the authors' experiments on the mash of carotene-sweet potato, varying as the thickness of the mash layer, the reaction of peroxidase did not become negative unless the sample received at least 20 minutes' heat-treatment.

The results obtained on the $CO_2$ gas stored sample, that the ratio of carotene-remaining was higher in the raw sample than in blanched, may be ascribed to that the ratio of stabilization with $CO_2$ gas surpassed the ratio of destruction due to heat-treatment.

## B. Enzymes affecting the decomposition of sweet potato-carotene

a) Preliminary experiment: Raw carotene-sweet potato was mashed and about 30 to 40 gm. each of it was precisely weighed in two flasks. A little toluene was added to the one of the flasks immediately and to the another after the steaming for 20 minutes. The carotene content of both samples were determined after being kept for 8 to 48 hrs. in an incubator regulated at 37°C.

Apart from these samples, it was inspected whether KCN has any inhibition to the carotene-destructive enzyme. That is, 10 cc. of 1% KCN was added to 30 gm. of mash, and then after incubation at 37°C for 48 hrs., it was compared with the non-added sample. The results of these experiments, related to the enzymatic action, are as in Table 2, 3 and 4.

Table 2.

Destruction of carotene in the raw sweet potato mash (37°C)

| Incubated hrs. | Moisture % | Carotene γ% | Ratio of remains % |
|---|---|---|---|
| 0 | 72.57 | 4117 | 100 |
| 8 | — | 3764 | 91 |
| 24 | — | 3570 | 87 |
| 48 | — | 2695 | 66 |

Table 3.

Destruction of carotene in the raw and steamed sweet potato mash (37°C)

| Incubated hrs. | Moisture % | Raw mash | | Steamed mash | |
|---|---|---|---|---|---|
| | | Carotene γ% | Ratio of remains % | Carotene γ% | Ratio of remains % |
| 0 | 64.54 | 6044 | 100 | 5780 | 100 |
| 24 | — | 4647 | 77 | — | — |
| 48 | — | 4426 | 73 | 5560 | 92 |

Table 4.

Inhibition to the enzymatic action with KCN (37°C, 24 hrs.)

| | Moisture % | Carotene γ% | Ratio of remains % |
|---|---|---|---|
| Original sample | 66.19 | 6009 | 100.0 |
| KCN added | — | 5984 | 99.6 |
| Non added | — | 4733 | 79.4 |

These results are brought together as follows:— (1) When raw mash was left at 37°C for 48 hrs., degree of carotene-destruction amounted to 27 to 34%. (2) In the case of the mash steamed and then treated likewise, the decrease was limited in 8%. (3) The destruction of carotene in raw mash was perfectly checked with addition of KCN. From these facts, the existence of enzyme affecting the decomposition of carotene, can be perceived.

b) Preparation of enzyme which takes parts in the degradation of carotene: Carotene-sweet potato, weighing 220 gm., was mashed and extracted with 100 cc. of acetate buffer solution (pH=4.5), and then the resultant juice, 150 cc. (pH=5.3), was centrifuged, and precipitated

starch being removed. The pH value was adjusted to 6.7 with ammonia, and then the precipitate with addition of 3 cc. of basic lead acetate solution was centrifuged, 37 gm. of $(NH_4)_2SO_4$ being added to the supernatant solution, impure material being removed. 50 gm. of $(NH_4)_2SO_4$ was again added, water being poured until no indissolved sulfate did remain. By centrifuging the solution of 190 cc. thus obtained, enzyme was precipitated and it was dissolved in 50 cc. of water, warmed at 63°C for 5 minutes and then resultant inactive precipitate was removed likewise. The supernatant solution, 95 cc. (pH=5.8), obtained in this way, was used as enzyme solution.

c) The action of the enzyme preparation on the carotene of sweet potato: Carotene-sweet potato, weighed about 300 gm., was mashed after 50 minutes' steaming, on which the enzyme solution was brought to act at 37°C for 24 hrs. The experimental results on the enzymatic degradation of carotene are tabulated in Table 5.

Table 5.

The enzymatic action on the carotene of sweet potato (37°C, 24 hrs.)

| Sample | Wt. of sample gm. | Vol. of enzyme solution cc. | Carotene γ% | Ratio of remains % |
|---|---|---|---|---|
| Just after steaming* | 20 | — | 5880 | 100 |
| Enzyme added | 30 | 10 | 4984 | 85 |
| 〃 | 30 | 10 | 4812 | 82 |
| Non added | 30 | (water 10) | 5815 | 99 |

* Moisture 71.91%

It can be comprehended from Table 5, that the enzyme solution which was prepared from sweet potato after the manner that Theorell *et al.*[22] separated lipoxidase from soy bean, participated in the destruction of sweet potato-carotene. Nevertheless, enzyme preparation here obtained was not so pure that it was not able to assert that this action was due to the existence of the lipoxidase itself.

## Summary

The results of these experiments on the destructive factors to the carotene of sweet potato are summarized as follows:—

1) Carotene in sweet potato is destructed with heat, light, air and enzyme.

2) Carotene in sweet potato mash decreases by about 5% during 20 minutes' blanching, and during this process the peroxidase loses its activity.

3) When sweet potato flour is made by dehydration after the blanching of sweet potato for 30 minutes, carotene decreases by about 7%.

4) The decreasing ratio of carotene in the sun-drying process is higher than in the dehydration.

5) Irradiation of ultraviolet ray remarkably decomposes carotene.

6) Atmospheric oxygen is one of the destructive factors to carotene, while $CO_2$ gas has preventive action on the decomposition of it.

7) Blanching process prior to the dehydration of sweet potato, effectively preserves more carotene than the untreating process.

8) Carotene is greatly destructed when the raw mash is incubated at 37°C, whereas, in the steamed mash, the loss of carotene remains in a little quantity, and moreover with addition of KCN to the raw mash, the decomposition can be perfectly checked.

9) The authors prepared an enzyme preparation from sweet potato and recognized that with the action of it on the steamed mash at 37°C for 24 hrs., 15 to 18% of carotene was destroyed.

From these results the authors concluded that this enzyme preparation is a seriously destructive factor against the carotene of sweet potato.

## III. On the Carotene-destructive Enzyme in Sweet Potato

Kôtaro NISHIDA and Yoshio YAMAMOTO

The enzyme, lipoxidase, which takes part in the destruction of carotene is observed only in the plant kingdom. Especially in soy bean,[16] the content of this enzyme is very high, and also in various kinds of beans besides soy bean,[30] and in alfalfa,[31] potato,[32] radish,[33] asparagus, wheat embryo, and pea-nut[34] etc. rather high quantities are found.

The authors pointed out in the foregoing report the existence of carotene-destructive enzyme in sweet potato, however, could not confirm its true feature. Fukuba,[35] afterwards, claimed that lipoxidase does not exist in sweet potato, and Mallette *et al.*[11] also did not find it in sweet potatoes. Sumner[36] mentioned that the highly activated peroxidase preparation, got from a horse-radish, accelerates the oxidation of carotene, and according to the explanation by Miller,[13] the failure in inactivating the enzyme prior to the dehydration process results in comparatively much carotene-loss during the storage of dehydrated sweet