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

potato. Therefore, even though the existence of lipoxidase would be denied, the possibility is well founded that there exists the enzyme which takes part in the degradation of carotene. For this reason, independently from lipoxidase, the authors inspected the general characters of the carotene-destructive enzyme which was pointed out in the foregoing report, and whether peroxidase and oxidase which are supposed to have the closest relations to the enzyme are really able to destruct the carotene or not.

Experiments and results

A. Experimental method

a) Sample of carotene: In order to get a homogeneous sample, carotene-sweet potato was peeled, sliced crosswise, steamed for 30 minutes and mashed, and the sample thus obtained was used as the substrate.

b) Enzyme solution: Carotene-sweet potato was mashed, the tissue of which was well broken in a mortar with quartz sand, and then five times water was added and centrifuged. The solution, filtered off from this supernatant with filter pulp, was used as crude enzyme solution.

Though we could not use the same sample and the same enzyme solution in all the experiments, in one series of the experiments, the constant sample and enzyme solution were used, and further, considering the catalytic action other than the enzymatic action, the experiments were carried out also on the control in which the crude enzyme solution was used after being boiled for 20 minutes.

c) Buffer solution: The McIlvaine's buffer was used.

d) Treatment: 20 gm. of sample, 10 cc. of enzyme solution or control solution and 1 cc. of toluene each were put into a conical flask of 100 cc. volume and homogeneously mixed; then after incubating for definite periods at a certain temperature, carotene contents were estimated.

B. Experimental results

a) Influence of pH value: The influence of the pH values ranging from 2.2 to 7.6 at 25°C and 37°C for 19 and 24 hrs. on the carotene contents are presented in Table 6 and Fig. 1. The optimum pH values for this enzymatic action were ranging from 5.5 to 6.0, and at any values outside this range, especially on alkaline side, the enzymatic actions were rapidly weakened.

Table 6-a.

The influence of pH value on the enzymatic action (25°C, 19 hrs.)

	pH	2.2	3.4	5.0	5.6	6.2	6.6	7.6
Control	Carotene (γ%)	4760	4783	—	4779	—	4802	4827
	Ratio of remains (%)	98.6	99.1	—	99.0	—	99.5	100
Enzyme added	Carotene (γ%)	4682	4466	4131	4054	4103	4422	4785
	Ratio of remains (%)	97.0	93.0	86.0	84.0	85.0	92.0	99.0

Table 6-b.

(37°C, 24 hrs.)

	pH	3.4	5.6	6.2	7.0
Control	Carotene (γ%)	5684	5715	5740	5800
	Ratio of remains (%)	98.0	98.5	99.0	100
Enzyme added	Carotene (γ%)	5509	4756	4925	5716
	Ratio of remains (%)	95.0	82.0	85.0	98.5

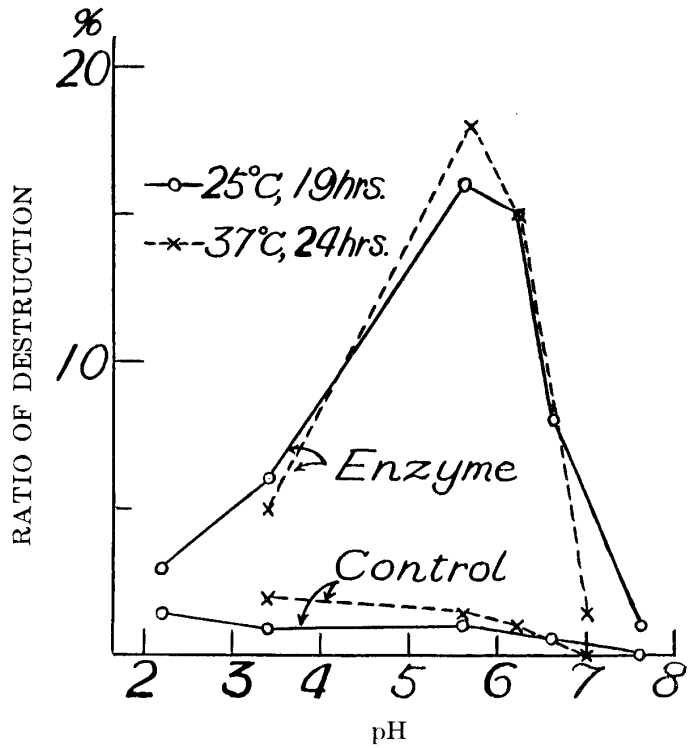

Fig. 1. The influence of pH value on the enzymatic action

b) Influence of temperature: Since the optimum pH value for this enzymatic action was concluded as ranging from 5.5 to 6.0, in the subsequent experiments the authors adopted 5.6 as pH value. The optimum temperature for this enzyme at pH 5.6 and 20 hrs. was between 25° and 30°C as shown in Table 7 and Fig. 2.

Table 7.

The influence of temperature on the enzymatic action (pH 5.6, 20 hrs.)

	Temp. °C	10	15	25	30	37	45
Control	Carotene (γ%)	6700	6633	—	6566	—	6499
	Ratio of remains (%)	100	99	—	98	—	97
Enzyme added	Carotene (γ%)	6635	6365	5494	5561	6097	6365
	Ratio of remains (%)	99	95	82	83	91	95

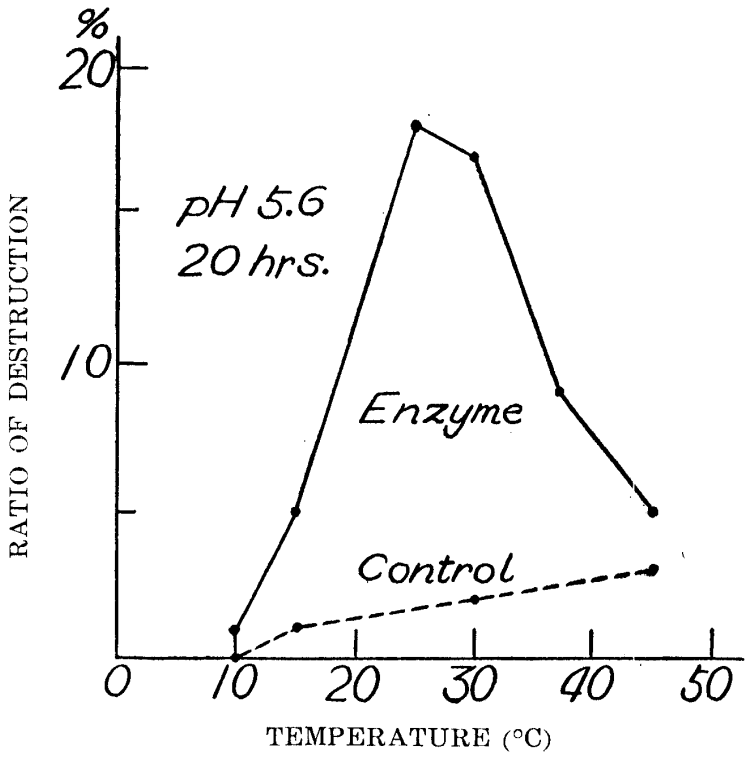

Fig. 2. The influence of temperature on the enzymatic action

c) Influence of reacting time: According to the results at pH 5.6 and 26°C, presented in Table 8 and Fig. 3, the action of this enzyme did not decrease from 10 hrs. till about 30 hrs., nevertheless whereafter decreased.

Table 8.

The influence of reacting time on the enzymatic action (pH 5.6, 26°C)

		Reacting time hrs.					
		0	10	20	30	40	50
Control	Carotene (γ%)	7011	6940	—	6870	—	6787
	Ratio of remains (%)	100	99	—	98	—	97
Enzyme added	Carotene (γ%)	7011	6800	6204	5609	5441	5260
	Ratio of remains (%)	100	97	89	80	78	75

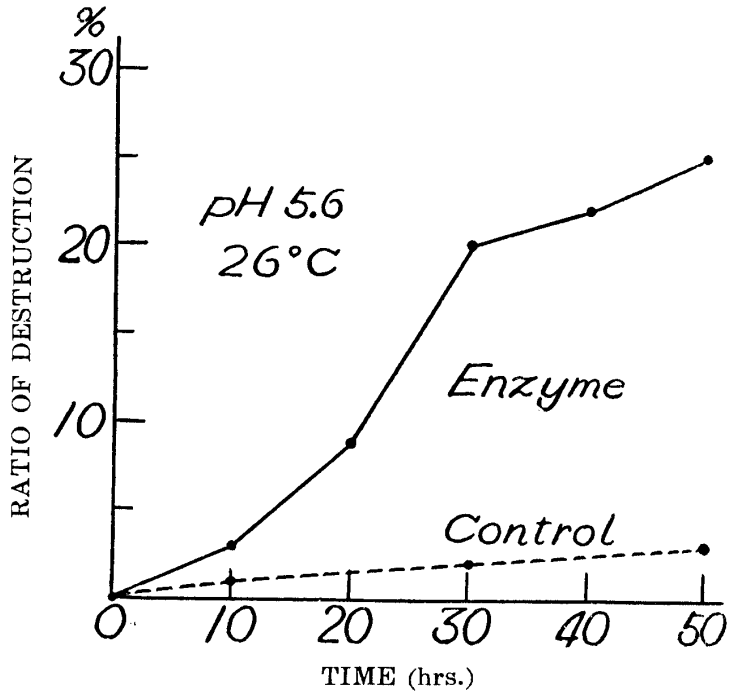

Fig. 3. The influence of reacting time on the enzymatic action

d) Thermostability: The enzyme solution was immersed in a water bath of definite temperature for 5 minutes and then it was treated in

Table 9.

The thermostability of the enzyme (pH 5.6, 27°C, 25 hrs.)

	Heating temperature (°C)			
	60	80	100	Control
Carotene (γ%)	5695	6432	6659	6700
Ratio of remains (%)	85	96	99	100

the above mentioned manner at 27°C for 25 hrs. According to the experimental results, this enzyme was considerably inactivated when heated at 80°C for 5 minutes (Table 9).

e) Inhibition due to KCN: The inhibition due to KCN to this enzymatic action was inspected in the concentration range of N/1000 to N/100000. As shown in Table 10, the enzymatic actions at pH 5.6, 36°C and 26 hrs. were almost perfectly checked with the existence of N/10000-KCN.

Table 10.

The inhibition of enzymatic action due to KCN (pH 5.6, 36°C, 26 hrs.)

	Concentration (N)			
	Control	1/1000	1/10000	1/100000
Carotene (γ%)	5800	5794	5748	5394
Ratio of remains (%)	100	99.9	99.1	93.0

Considering from these experimental results, it is very probable that the active center of this enzyme would be a sort of heavy metal. But on the other hand, Irvine *et al.*[37] reported that KCN does not inhibit the action of pure lipoxidase, but when lipoxidase is not pure it does. Therefore, further inspections should be carried out in order to testify the relations between lipoxidase and the enzyme that the authors pointed out to be participating in carotene-destruction.

f) Study on enzyme in comparison with soy bean-lipoxidase: With an idea that one of the means clarifying the relation between this enzyme and soy bean-lipoxidase might be to investigate and compare the destructive actions against carotene which exist between two enzymes, the authors prepared crude lipoxidase and treated in the same conditions as the experiments mentioned above. 2 gm. of defatted

Table 11.

The action of the soy bean-lipoxidase on the sweet potato-carotene (pH 5.6, 27.5°C, 26 hrs.)

	Control	Enzyme solution from		
		Sweet potatoes		Soy bean
		Hayato*	Nôrin No. 2**	
Carotene (γ%)	6673	5539	6442	4805
Ratio of remains (%)	100	83	97	72

* Carotene-sweet potato. ** Non carotene-sweet potato

soy bean meal was suspended in 50 cc. of water and centrifuged, the supernatant solution thus obtained being used as crude enzyme. The results in these experiments are as shown in Table 11.

According to this table, in the case of soy bean the ratio of destruction amounted to 28%, which is far away higher than in sweet potatoes and is recognized as being due to lipoxidase.

It is also interesting that the ratio of destruction is higher in the Hayato variety of sweet potato than in the Nôrin No. 2, but be it as may, it is to be deduced that there is an essential difference between the action of enzymes of sweet potatoes and that of soy bean.

In this place, the hitherto reported studies[11,15] on the action of peroxidase and oxidase etc. on carotene could be summarized as follows: some investigator said that they had no direct relations; and the other said that they had non action; and thus constant conclusion have not yet been acquired. Therefore, the authors inspected the action of peroxidase on carotene as the first step.

g) The action of peroxidase on the sweet potato-carotene: The preparation of peroxidase was carried out after the Kondô's method.[38] 1 kg. of sweet potato was germinated at 27°C for one week, then mashed and with water squeezed, thus 480 cc. of juice (G.N. 0.6) was obtained. The precipitate, starch, was removed as much as possible by decantation and the enzyme was precipitated with 0.9-saturation of $(NH_4)_2SO_4$ to the supernatant. This brown precipitate was dissolved in 150 cc. of water, from which with 0.4-saturation of $(NH_4)_2SO_4$ the impure materials were precipitated. To the filtrate, $(NH_4)_2SO_4$ was further 0.75-saturated, and enzyme was again precipitated, which was suspended in water and dialyzed for 48 hrs. with distilled water. From the dialyzate, 30 cc. here obtained, impure proteins and pigments etc. were absorptively removed with addition of newly prepared suspension of $Ca_3(PO_4)_2$ and then centrifuged. The supernatant (G.N. 670), 28 cc., was used as the enzyme solution of peroxidase (activity: about 1,100 times). The results in the treatment at pH 5.6, 26°C and 24 hrs. were as in Table 12.

Table 12.

The action of peroxidase on the sweet potato-carotene (pH 5.6, 26°C, 24 hrs.)

	Control	Peroxidase from	
		Hayato	Nôrin No. 2
Carotene (γ%)	6620	6135	6200
Ratio of remains (%)	100	93	94

In these results, two peroxidase preparations got from both Hayato and Nôrin No. 2 destructed carotene with scarcely any differences and the ratio of destruction was 6 to 7%. Therefore, we can guess that peroxidase is one enzyme that to some extent takes part in the destruction of carotene.

h) Experiments with oxidase model: As the model of an oxidase in our laboratory synthesized chloropentammine cobaltic chloride[3] was used. This cobaltic complex salt gives greenish or brownish black coloration to guaiacum tincture or pyrogallol and moreover, this coloration is inhibited with $HgCl_2$. Thus, it is recognized as a sort of enzyme model to be analogous to the oxidase in its action.

Preparation of substrate: Referred to the method by Willstätter *et al.*[39] and Strain,[40] the petroleum etheral (bp. 60°C) solution of carotene was condensed in vacuum, and then was made into the acetone solution (1697 γ%).

Firstly, to know the optimum pH value of oxidase model to carotene, following preliminary experiments were carried out. 0.5 cc. of McIlvaine's buffer solution (pH 4 to 8), 0.5 cc. of carotene solution, 4 cc. of acetone and 2 cc. of M/500 cobaltic complex salt solution or control solution (M/1000 $HgCl_2$ added to the complex salt solution) were well mixed and the changes of the color at room temperature were qualitatively compared. In these results (Table 13) the discoloration was maximum at pH 7 to 8, and so pH value as 7.0 was adopted in the subsequent experiments.

Table 13.

The influence of pH value on the oxidase model

Time \ pH	4.0	5.0	6.0	7.0	8.0
Just after added	Pale yellow ++	Pale yellow ++	Pale yellow ++	Pale yellow ++	Pale yellow ++
After 60 min.	++	++	++	+	+
After 120 min.	Pale greenish yellow +	Pale greenish yellow +	Pale greenish yellow +	Colorless ±	Colorless ±

++, +, ±: Show the depth of coloring, in order from dark to light. In the control solution, almost no difference was observed with the change of pH value, and also no change in its pale yellow color due to standing was recognized.

Then the effect of concentration of carotene solution on the oxidase model at pH 7.0 was inspected and the results are shown in Table 14.

Table 14.

The effect of concentration of carotene solution on the oxidase model

Concn. (cc.) / Time	0.3	0.5 (68 γ%)	0.7	1.0
Just after added	Pale yellow ++	Pale yellow ++	Pale yellow ++	Yellow +++
After 60 min.	+	+	++	+++
After 120 min.	Colorless ±	Colorless ±	++	+++

In the control solutions, no discoloration was observed in each concentration.

According to Table 13 and 14, it will be seen that this oxidase model, also can act in the case of very dilute carotene solution. In other words, it may be deduced that the oxidase has possibility to destruct carotene, though slightly.

Discussion

Considering from above mentioned experimental results, we may suppose that the enzyme which the authors pointed out to have relations to the degradation of sweet potato-carotene, is not probably lipoxidase itself. As to the propriety of this deduction, the authors could give assurance to some extent by the carotene-destructive ability of peroxidase and oxidase. However, in contrast to the ratio of destruction with the enzyme solution from carotene-sweet potato, amounting to 15 to 18%, the ratio of destruction with peroxidase showed only 6 to 7%, and further, the peroxidase preparation the authors employed, was difficult to be rendered as pure, nevertheless, showed high activity.

Deducing from these facts, the authors can not conclude that the enzymatic destruction of carotene, the authors pointed out, is merely due to the individual action of peroxidase or oxidase. Mallette *et al.*[11] pointed out in sweet potato, the existence of such oxidases as catecholase, cresolase, laccase etc., and therefore it may be reasonable to conclude that it is ascribed to the co-operated action of these enzymes.

Summary

(1) The existence of enzyme, affecting the destruction of sweet potato-carotene, was confirmed in the carotene-sweet potato and the differences between this enzyme and the soy bean-lipoxidase were pointed out.

(2) The optimum pH values for this enzyme were from 5.5 to 6.0

and the optimum temperatures for pH 5.6 and 24 hrs. incubation were 25° to 30°C.

(3) At pH 5.6 and 26°C, the enzymatic action decreased after 30 hrs., and was inhibited to some extent when heated at 80°C for 5 minutes.

(4) The enzyme activity was inhibited when the concentration of KCN was over N/1000.

(5) In the comparison between the ratios of carotene-destruction respectively with sweet potato-enzyme solution and with soy bean-enzyme solution at pH 5.6, 27°C and 26 hrs., the destruction with latter amounted to 28%, while with former 17% in Hayato variety and 3.5% in Nôrin No. 2 variety.

(6) It was verified that the action of carotene-destroying enzyme is mainly due to peroxidase, and an idea that this action, is due to the co-operative actions of both peroxidase and several oxidases was assumed as proper.

IV. Trial Brewing of the Carotene-enriched Miso (Fermented Bean Paste)

Kôtaro Nishida and Yoshio Yamamoto

Recently the problems of enriched foods have been taken up with great concerns and, among these as regards to the enriched Miso, there are studies on various kinds of Miso, such as Vitamin B_2-enriched Miso,[41,42] carotene-enriched Miso with micro-organisms[43,44] and moreover calcium-, animal protein- and Vitamin B_1-etc. enriched Miso.[45]

Now, for the application of carotene to the food industry, it is neccessary to keep the carotene as much aloof as possible from the destructive factors such as light, oxygen, temperature, moisture and enzyme etc. The authors have already pointed out these facts in the previous reports, yet it is generally recognized that carotene is lost during storage. To prevent this destruction, many kinds of antioxidants have been examined. They are in numerous number,[46] however, when classified after their functional groups almost of them may principally belong to aromatic amine or hydroxy compounds. Moreover lately, some of sulfur containing compound[47,48] have been reported as effective. All of these antioxidants, however, can not be simply expected as practically valuable because of risks infringing the food hygiene disciprinary regulations.