IV. Trial Brewing of the Carotene-enriched Miso : Fermented Bean Paste(Studies on the Stability of Carotene in Sweet Potatoes)

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.

The authors brewed carotene-enriched Miso as an utilization of the carotene-sweet potato and inspected the effects on the stability of carotene with addition of several substances practically available, excluding diphenyl amine.

Experiments and results

The sample of Miso was mashed on Sept. 2, 1951, the main materials used and treatment of them are as follows:—

a) Rice: weight (7.5 kg.), steeped (20°C, 14 hrs.), steamed (at 100°C, 1.5 hrs.), *Kôji*-making (usual method. weight finished: 7.8 kg.)

b) Soy bean: weight (13 kg.), steeped (20°C, 16 hrs.), steamed (at 100°C, 4 hrs., weight steamed: 27.4 kg.)

c) Carotene-sweet potato: weight (7.5 kg.), treatment (steamed for 50 min., peeled, fibrous material removed as possible, homogeneously mashed. weight finished: 7.1 kg.)

The mashing was followed to the usual method and the trial brewing was divided in six groups. The ratio of materials and the kind and quantity of annexes are shown in Table 15.

In order to inspect the effects of annexes, the authors examined the vicissitudes of carotene contents during the ripening period of Miso in these six trial brewing groups (Table 16).

Table 15.

Tne composition of trial brewing of Miso

No.	Group	Annex	Mashing ratio	Remarks
1	Control	0gm.	Soy bean: 1.5 kg. White rice: 0.75 〃 NaCl: 0.50 〃 Sweet potato: 0.75 〃 Water: 300 cc.	In a jar (10L. volume), covered with cellophane, lidded and ripened at room pemperature.
2	Ca-lactate	39 (1.0%)	Same as above	Annex was blended homogeneously with sweet potato. Others, same as above.
3	Much NaCl	400	Same as above	Same as above
4	Barleygerm oil	32 (0.8%)	Same as above	Same as above
5	Diphenyl amine	15 (400 mg%)	Same as above	Same as above
6	Yeast	375 (9.0%)	Same as above	Same as above

Figures in the parentheses show the percentages to the total material.

Table 16.

The vicissitudes of carotene content accompanied with ripening of Miso

No.	Group	Carotene	Ripening days			
			0	15	35	70
1	Control	In Miso (γ%)	674	662	643	613
		In dry matter (γ%)	1529	1500	1460	1390
		Ratio of remains (%)	100	98	96	91
2	Ca-lactate	Same as above	674	612	542	425
			1518	1378	1220	959
			100	91	80	63
3	Much NaCl	Same as above	682	602	499	344
			1438	1270	1053	725
			100	88	73	50
4	Barleygerm oil	Same as above	675	574	536	456
			1515	1376	1201	1025
			100	85	79	68
5	Diphenyl amine	Same as above	670	664	660	612
			1524	1510	1500	1393
			100	99	99	91
6	Yeast	Same as above	626	610	563	459
			1435	1400	1291	1052
			100	97	90	73

According to these experimental results, the ratios of remaining carotene in the enriched Miso, finished 70 days' ripening, were as follows:—more than 90% both in the diphenyl amine added Miso and in the control, 73% on yeast, 63% to 68% on barley-germ oil and Ca-lactate, and 50% on much NaCl.

In addition, when ripened for 35 days, the ratios of remains were more than 80% except rich-in-NaCl Miso, and such high retentions especially in the case of diphenyl amine as 99%, and in control as 96% were observed.

Discussion

Diphenyl amine has been generally recognized as eminently effective antioxidant[49] and it may be no wonder that it has shown the best results in the authors' experiments, too. The authors, however, did not expect such results that almost equal retention was observed in the control as in this case. The studies in future are expected on the reason of these facts, yet they are considered as very interesting from the standpoints of utilization and storage of sweet potato-carotene.

The yeast was added from the objection to inspect the effect of special components such as Vitamin B-complex or nucleic acid etc., but

rather, they have probably accelerated the oxidation of carotene. Such tendency was further remarkable in the existence of barley-germ oil or Ca-lactate, and the promotion of enzymatic action may be counted up as one of the causes.

Speaking of the details, the chemical changes in Miso during the ripening period is very complex, and Fukuba[35] pointed out the existence of lipoxidase in several fungi, and the authors recognized that peroxidase also destructs carotene. As the explanation of the above an acceptable deduction may be that the effective components in the bread yeast (does not possess lipoxidase) and barley-germ oil or Ca-lactate etc. promoted the enzymatic action. Or otherwise, further different reasons would be counted up. On all these points in particular, the authors are desirous to investigate further.

On the effect of Vitamin E, moreover, according to H. L. Mitchell *et al.*[50] it is known that Vitamin E alone is not so much effective on the stability of condensed carotene. P. L. Harrs *et al.*[51] reported that condensed carotene is more stable than crystalline carotene dissolved in the same oil as former, since Vitamin E intermixes in the condensate. An argument on Vitamin E and existence of another synergist was also reported. However, in this experiment, the effect of germ oil as anti-oxidative annex was not recognized.

Sodium chloride promotes the decomposition of Vitamin A according to the studies on its effect by Ariga,[52] and the same effect may be expected also on carotene. In the authors' experiments, too, much quantity of NaCl remarkably increased the destruction of carotene, and for this reason, in the case of brewing of carotene-enriched · Miso it is neccessary to pay attention to the quantity of NaCl to be used.

Summary

(1) The carotene-enriched Miso was brewed with the carotene-sweet potato on a trial and at the same time, to it several substances such as Ca-lactate, NaCl, barley-germ oil, diphenyl amine and steamed yeast were added, in order to examine the effect of these materials on the carotene retention during its ripening period.

(2) Of the materials tested, only diphenyl amine had the maximum stabilizing effect, whereas, its carotene retention in control was almost equal to the case of diphenyl amine where more than 90% of carotene remained after 70 days.

(3) From the standpoint of utilization and storage of sweet potato-carotene, we believed that it is one of the most suitable methods to

use the carotene-sweet potato for manufacturing carotene-enriched Miso, since the carotene content in control is scarcely reduced even though no effective material is added.

V. On the Substances that Prevent the Oxidation of Sweet Potato-carotene

Kôtaro NISHIDA, Yoshio YAMAMOTO, Akira KOBAYASHI and Kazuko YAMAMOTO

It has been argued[51,53,54] that there exist some substances in plant cells together with carotene and these prevent the oxidation of it. The authors also pointed out in the previous report that the carotene in steamed sweet potato was unexpectedly stable, though did not discuss on its reason. Yet, tannin materials are those presumed to be the most probable antioxidant for sweet potato-carotene, and so the authors undertook the investigations on the abilities of the sweet potato-tannin which prevents the oxidation of carotene.

Recent studies by Bickoff[55] showed the effectiveness of catechol derivatives as phenolic antioxidants for the mineral oil solution of carotene. In the study on the sweet potato-tannin, Ose *et al.*[56] reported that it belongs to catechol tannin, but not on details. Moreover lately, Nelson *et al.*[57] isolated chlorogenic acid, coffeic acid and the closely related compounds to them from sweet potato with counter current distribution method and reported the minute experimental results on the respiration of sweet potato. It is interesting, too for the problem of antioxidants that the existence of these substances was proved.

Experiments and results

A. Preparation of tannins from sweet potato and coffee

a) Preparation of sweet potato-tannin: Raw carotene-sweet potato was mashed and at once thrown into boiling ethanol, extracted there, and then filtered. Then ethanol was added to the residue and extraction was repeated in the same way. When the $FeCl_3$-reaction on the extract turned negative, the combined extracts were condensed in partial vacuum of CO_2 gas current, yellowish brown colored sticky material being obtained. To this material water was added and with ether-treatment for several times, ether soluble part was removed. The residue of ether-extraction was reextracted with ethyl acetate;