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;

ethyl acetate portion was washed three times, dehydrated with anhydrous sodium sulfate, and then condensed in partial vacuum of CO_2 gas current. White curd-like precipitate, gotten with addition of chloroform to the condensate, was spread on a clay-plate and dried in vacuum. In this way sweet potato-tannin was acquired as yellowish white powder.

b) Preparation of coffee-tannin: Coffee-tannin was prepared from raw coffee bean in accordance with above mentioned manner. Thus obtained is crude tannin in which chlorogenic acid is a main component, soluble in water or ethanol, insoluble in ether, and gives such colorations that green color with ammonia, reddish brown with NaOH and greenish black with $FeCl_3$.

B. Experiments on the steamed carotene-sweet potato

a) Effect of NaCl on the destruction of carotene: The authors recognized in the foregoing report on the brewing of enriched Miso that much NaCl promotes the oxidation of carotene; while in the case of storing the steamed carotene-sweet potato, for preventing its rottenness addition of NaCl is considered as the most suitable method practicable. Moreover, since it is thought to be desirable, in the studies lasting long days, to experiment with addition of NaCl on one hand for preventing rottenness and on the other for applying its action promoting the destruction of carotene, experiments were executed on the effect of concentration of NaCl to be added. The results are shown in Table 17.

Table 17.

Effect of NaCl on the destruction of carotene (20°C)

Concn. of NaCl (%)	0 Days	5 Days		15 Days	
	Carotene (γ%)	Carotene (γ%)	Ratio of remains (%)	Carotene (γ%)	Ratio of remains (%)
5	5310	5310*	100*	—	—
10	5040	5040	100	4370*	87*
15	4820	4720	98	4170	87
20	4510	4330	96	3900	86
30	3940	3610	92	3420	87

* Moulded

According to this table, when the concentration of NaCl was 15 to 30%, more carotene was destructed after 5 days as NaCl increases, while after 15 days, the retention of carotene did not differ so much. However, in case NaCl was under 10%, steamed sweet potato was

moulded and could not be stored more than 5 days and so it is required for the long period storage that the concentration of NaCl is at least about 15%.

b) Antioxidative ability of tannins on the sweet potato-carotene: In the results of experiments on the antioxidative effect of sweet potato-tannin on the steamed carotene-sweet potato (carotene content: 3940 γ%), its effect was evidently recognized when tannin was added as much as 0.33% as in Table 18.

Table 18.

Antioxidative effect of sweet potato-tannin on the carotene (NaCl 30%, 20°C)

Concn. of tannin (%)	5 Days		15 Days	
	Carotene (γ%)	Ratio of remains (%)	Carotene (γ%)	Ratio of remains (%)
0 (Control)	3610	92	3400	86
0.06	3630	92	3420	87
0.33	3920	99	3720	94

When comparison is made between the coffee-tannin and the sweet potato-tannin in their respective antioxidative effect on the steamed sweet potato (carotene content: 7790 γ%), the former surpassed the latter as apparently shown in Table 19.

Table 19.

Comparison of antioxidative effect of coffee- and sweet potato-tannin on the carotene (NaCl: 30%, 30°C, 8 days)

Tannin	Concn. of tannin (%)	Carotene (γ%)	Ratio of remains (%)
Control	0	4200	54
Sweet potato-	0.2	4540	58
Coffee-	0.2	4930	63

C. Antioxidative ability of tannins for the solution of carotene

The antioxidative ability of tannins was examined by determining the carotene decompositions, in a carotene solution in mixture of acetone and ethanol in 2:1 ratio,[18] respectively due to the autoxidation, H_2O_2, and sweet potato-enzyme.

a) Antioxidative effect of tannins on the autoxidation of carotene:

Carotene solutions were left respectively for 10 days at 20°C, and for 13 days at 30° and 40°C, the effects of the two tannins added therein were compared and the results are presented in Table 20.

Table 20.

Antioxidative ability of tannins on the autoxidation of carotene

Tannin	Concn. of tannin %	20°C, 10 days		30°C, 13 days		40°C, 13 days	
		Carotene γ%	Ratio of remains%	Carotene γ%	Ratio of remains%	Carotene γ%	Ratio of remains%
Stock solution	—	2200	100	3517	100	4136	100
Control	0	2120	96	2426	69	2011	49
Sweet potato-	0.12	2130	97	—	—	2155	52
Coffee-	0.12	2180	99	2677	76	2260	55

As perceived from this table, in the column at 20°C for 10 days, both these two tannins gave scarcely any difference to the stability of carotene, when compared with the control. That is, carotene is stable in this case. At higher temperature, however, its ratio of destruction also becomes higher and at the same time, the antioxidative ability of tannins is apparently recognized.

b) Antioxidative ability of tannins for the decomposition of carotene due to H_2O_2: According to the foregoing experiments, carotene in solution is fairly stable at the comparatively low temperature; therefore, the antioxidative ability of tannins was examined in such a case that H_2O_2 was added to the carotene solution in order to strengthen the oxidation conditions.

i) Destruction of carotene due to H_2O_2: Firstly, the following treatments were executed to determine the ratio at which carotene

Table 21.

Destruction of carotene due to H_2O_2 (30°C, 96 hrs.)

Dilution times of H_2O_2	Concn. of H_2O_2 in solution %	Carotene γ%	Ratio of remains %
—	0 (Control)	3745	97
10 times	0.29	3545	92
8 〃	0.36	3111	81
6 〃	0.48	2744	71
4 〃	0.72	2345	61
2 〃	1.44	1744	46
1 〃	2.89	809	21

was decomposed, that is, 31.8% H_2O_2 was divided and diluted in various dilution ratios, such as 2, 4, 6, etc., and each was added to the carotene solution prepared from sweet potato (3845 γ%), and after being left at 30°C for 96 hrs., remains of carotene were determined. The results are shown in Table 21 and Fig. 4.

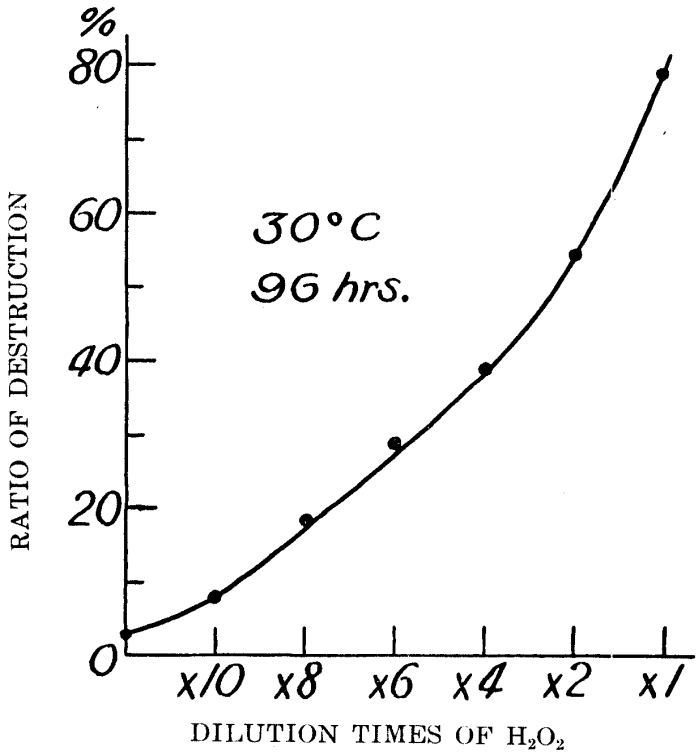

Fig. 4. Destruction of carotene due to H_2O_2

ii) Antioxidative ability of tannins for the decomposition of carotene due to H_2O_2: To 20 cc. of carotene solution, definite volume of alcoholic solution of tannin and then 2 cc. of H_2O_2 (31.8 wt. %) were added. Total volume was made up to 25 cc. in each of these flasks, which were tightly corked and left at definite temperatures and for definite periods respectively. The results that the carotene was estimated are shown in Table 22.

c) Antioxidative ability of tannin for the decomposition of carotene due to sweet potato-enzyme:

i) In the case where only sweet potato-enzyme was added: Carotene-sweet potato mash was extracted with water weighing half of the mash. The squeezed juice was centrifuged and then filtered with a glass filter; the filtrate thus obtained, was used as crude enzyme solution. Coffee-tannin and 1 cc. of sweet potato-juice were added to

Table 22.

Antioxidative ability of tannins on the decomposition of carotene due to H_2O_2

Tannin	No.	1		2		3		4	
	Temp.	about 10°C*		40°C		30°C		30°C	
	Time	24 hrs.		24 hrs.		72 hrs.		120 hrs.	
	Concn. of tannin	Carotene	Ratio of remains	Carotene	Ratio of remains	Carotene	Ratio of remains	Carotene	Ratio of remains
	%	γ%	%	γ%	%	γ%	%	γ%	%
Stock solution	—	4171	100	4171	100	3376	100	3517	100
Control	0	3019	72	1388	33	888	26	624	18
Sweet potato-tannin	0.03	—	—	—	—	993	29	—	—
	0.10	—	—	—	—	—	—	980	28
Coffee-tannin	0.03	—	—	—	—	1146	34	—	—
	0.10	3513	84	1459	35	—	—	—	—
	0.15	3576	86	1702	41	—	—	—	—

* In a refrigerator

20 cc. of carotene solution and was left at a definite temperature for a definite period. The results are as shown in Table 23.

Table 23.

Antioxidative ability of tannin on the decomposition of carotene due to sweet potato-enzyme

	Concn. of tannin %	30°C, 4 days		40°C, 5 days	
		Carotene γ%	Ratio of remains %	Carotene γ%	Ratio of remains %
Stock solution	—	4669	100	4136	100
Control	0	3979	85	2704	65
Coffee-tannin	0.05	4024	86	2876	70
	0.10	4298	92	—	—
	0.15	4408	94	3025	73

ii) In the case where sweet potato-enzyme and H_2O_2 were added: Carotene-sweet potato mash was extracted with McIlvaine's buffer (pH=5.6) weighing half of the mash in a mortar with quartz sand. Hereafter, the solution treated as above mentioned was used as enzyme solution. Coffee-tannin as 0.16%, 2 cc. of enzyme solution, 1 cc. of 3% H_2O_2 were added to 20 cc. of carotene solution and was left at 40°C for 6 hrs. The results in this case are shown in Table 24.

Table 24.

Antioxidative ability of tannin on the decomposition of carotene due to sweet potato-enzyme and H_2O_2 (40°C, 6 hrs.)

	Concn. of tannin %	Carotene γ%	Ratio of remains %
Stock solution	—	4554	100
Control	0	3649	80
Coffee-tannin	0.16	3802	83

Discussion

The authors recognized in the previous reports that carotene in steamed sweet potato or in carotene-enriched Miso with sweet potato was preserved in considerably stable state. These facts are expounded to be due not only to the fact that the enzyme — peroxidase — participating in the decomposition of carotene, was inactivated during heating process, but also to the antioxidative action of tannin materials that exist together with carotene in the cell of sweet potato.

In the consequence of experiments based on the above point of view, it was recognized that sweet potato-tannin apparently had these actions, and further, in the comparison of these actions with that of coffee-tannin, the latter proved to be more effective. This is perhaps due to the fact that coffee-tannin mostly consists of chlorogenic acid which is one of ortho hydroxy phenols and have antioxidative ability.

Chlorogenic acid is contained in sweet potato-tannin, too, nevertheless, its quantity is extremely small compared with coffee-tannin and this well explain the foregoing facts.

Summary

(1) Carotene in steamed sweet potato is fairly stable at the temperature as high as about 20°C, though the steamed sweet potato might become mouldy and impossible to be stored as it is. In order to prevent rottenness by means of NaCl-addition, it is sufficient to make its content about 15%.

(2) Antisepticizing the steamed sweet potato with addition of NaCl, the antioxidative ability of sweet potato- and coffee-tannin was examined and they both proved to be effective.

(3) When carotene solution was left to stand in natural state and tannins were used as antioxidants for its autoxidation, it was recognized that they were apparently effective and the coffee-tannin was always superior.

(4) The destructive actions of H_2O_2 on carotene were estimated and that the ratio of destruction increases in accordance with the concentration of H_2O_2 was observed.

(5) The effects of tannins were examined when H_2O_2 was added to the carotene solution as promoting agent for its decomposition.

(6) The antioxidative ability of tannin was examined, when sweet potato-juice was added to the carotene solution on which the enzyme influencing the decomposition of carotene was made to act. In this test, tannin was always recognized as effective.

(7) The influence of temperature on the stability of carotene solution was very eminent.

(8) In carotene-sweet potato there existed together with carotene some substances that prevented its oxidation. They were principally consists of sweet potato-tannin.

(9) As the important components that prevent the oxidation of carotene, the existence of ortho hydroxy compounds such as chlorogenic acid or coffeic acid etc. in the sweet potato-tannin may not be overlooked.

VI. On the Constitution of Sweet Potato-tannin

Kôtaro NISHIDA, Akira KOBAYASHI and Kazuko YAMAMOTO

The authors pointed out in the foregoing reports, the sweet potato-tannin as the substances which exist in sweet potato together with carotene and prevent its oxidation. On the constitution of this tannin, however, there has been reported scarcely any study.

Ose *et al.*[56] reported that the sweet potato-tannin was assumed to be grouped in the catechol tannin, since several qualitative reactions on it showed that of catechol tannin, being excepted the HCl-formaldehyde reaction. For the study on the constitution of the sweet potato-tannin, the authors adopted the paper chromatography on the potash fusion products of it. Paper chromatography is recently becoming to be applied also in the field of tannin investigations,[58)~62)] and among them Asquith,[61)] using new solvents, solved difficulties in the paper chromatography of the simpler phenols.

Experiments and results

a) Potash fusion of sweet potato-tannin: Potash fusion of sweet potato-tannin was executed after the manner which the authors had